BIOTIC PEST DAMAGE OF GREEN ALDER (*ALNUS FRUTICOSA*): SUSCEPTIBILITY TO A STEM DISEASE (*VALSA MELANODISCUS*) AND FUNCTIONAL CHANGES FOLLOWING INSECT HERBIVORY

By

Jennifer K. Rohrs-Richey

RECOMMENDED:

Advisory Committee Chair

Chair, Department of Biology and Wildlife

APPROVED:

Dean, College of Natural Science and Mathematics

Dean of the Graduate School

Dec 6, 2010

Date

BIOTIC PEST DAMAGE OF GREEN ALDER (*ALNUS FRUTICOSA*): SUSCEPTIBILITY TO A STEM DISEASE (*VALSA MELANODISCUS*) AND FUNCTIONAL CHANGES FOLLOWING INSECT HERBIVORY

A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

By

Jennifer K. Rohrs-Richey, M.S.

Fairbanks, Alaska

December 2010

ABSTRACT

Since the late 1990s, researchers have been predicting that a warming climate will lead to higher levels of plant disease damage. This appears to be the current trend in the boreal region; however, the level of complexity inherent to plant-pest interactions makes it difficult to make predictions across plant-pest systems. This study focuses on a boreal shrub in Alaska, *Alnus fruticosa*, which is currently a host to several insect and fungal pest species that are either already at epidemic status or have recently achieved epidemic status on other *Alnus* species in Alaska. Against the backdrop of a warming boreal forest, the overall aim of my study was to evaluate the response of *A. fruticosa* to two types of pest damage: the stem canker disease *Valsa melanodiscus* (anamorph *Cytospora umbrina*) and defoliation damage from insect leaf chewers. Our results indicate that, despite pest-related damage to the sapwood or leaf area, alders have physiological mechanisms in place to maintain homeostasis or recovery following disease damage. At the leaf-level, alders adjusted photosynthesis and stomatal conductance to cope with disease, despite decreased water transport and down-regulated light-response. At the ramet level, alders coordinated rates of water loss, hydraulic conductance, and maintenance leaf water balance following partial defoliation. These physiological host responses are not part of classical disease triangles, yet these types of host responses are likely to affect disease outcome in certain plant-pest systems and could potentially determine the trajectory of disease development.

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ACKNOWLEDGEMENTS

I would like to thank each of my committee members, who each brought a different, but complementary, skill set to this study. My main advisor, Christa Mulder, has a background in plant-herbivore interactions, a keen sense for experimental design and statistics, and is a scrupulous and thorough editor. Barbara (Bitty) Roy brought her expertise in the evolutionary ecology of plant-pathogen interactions to my project. Roger Ruess, an ecosystem ecologist, challenged me to put my small-scale study into a larger-scale perspective. Lori Winton, a plant pathologist, trained me in pathology techniques and conducting greenhouse experiments with pathogens. Donie Bret-Harte brought her expertise in plant physiology to my project. In addition to thanking my committee members, I would also like to thank statistics professor Ron Barry, for his advice on data analysis. Heather McIntyre, retired manager for the Institute of Arctic Biology greenhouse, was involved in all aspects of germinating, growing, and maintaining the plant material for my greenhouse experiments. Glen Stanosz, professor of forest pathology at the University of Wisconsin, was an honorary committee member. Glen trained me in field inoculation techniques and many other aspects of plant pathology. Gerard Adams, plant pathology professor at Michigan State University, donated the *Valsa melanodiscus* isolates that were used in this study. I would also like to thank my husband, Brent Richey, the Rohrs family, and the Richey family.

CHAPTER 1

GENERAL INTRODUCTION

In the mid to late 1990's, there was mounting evidence that climate change was underway but there was limited research to address the impacts of climate change on plant diseases in managed and natural systems (Coakley et al. 1999). Since then, researchers have considered how climate-related changes in plant-pest dynamics would affect agricultural production (Runion 2003, Anderson et al. 2004), economic costs of damage to the forest industry (Ayres and Lombardo 2000, Dale et al. 2001), and the impact of diseases on vegetation structure and function (Malmström and Raffa 2000). Whether disease is studied in an agricultural field, managed forest, or ecosystem, the disease outcome is a function of the interplay of the same factors: the host, the pest, and the environment. This is the classic disease triangle (Manion 1991), and consideration of its components have been fundamental to all studies of plant-pest dynamics (Fig.1.1).

Changes to the environmental component of the disease triangle will likely lead to changes in plant-pest interactions. For example, under a warming climate, higher temperatures are expected to directly affect pest biology, resulting in increased survival, reproduction, metabolism, and movement of pests (Dukes et al. 2009). Where insect herbivores become more metabolically active and mobile, there is increased risk of higher pest damage to the host (Dukes et al. 2009). Warmer temperatures are also expected to directly affect the host. High latitude climate changes often operate at a faster pace than the scale at which plants are able to migrate or adapt to the altered climate

(Jump and Penuelas 2005, Garrett et al. 2006). This may push plants beyond the physiological limits of their current ranges (Garrett et al. 2006), resulting in long-term exposure to stresses such as high temperature or low precipitation. Long-term, climate-induced stress can cause permanent strain to physiological functions that are essential for defending the plant against pests and recovering from disease damage (Panek and Waring 1997, Ayres and Lomabardo 2000).

In this dissertation, my focus is primarily on the host component of the disease triangle. The host component has been approached by researchers from a wide range of disciplines, including the fields of plant pathology, entomology, landscape ecology, and physiology. Each discipline focuses on different levels of organization and has slightly different perspectives on the role of the host in the disease triangle. Taken together, the different perspectives of the host can all be valuable for anticipating the ultimate disease outcome.

One of the most common perspectives of the host has been as a substrate for pest attack or colonization. For instance, plant pathologists have traditionally evaluated the suitability of the host for colonization in terms of moisture (Bloomberg 1962, Bier 1953), the histological response to infection (Biggs et al. 1983), morphological barriers to pathogen colonization, and features of host anatomy that facilitate disease development (Bloomberg and Farris 1963). These substrate-based studies are crucial for assessing host suitability for pest attack; however, they typically do not evaluate host responses to pest

damage outside of the immediate vicinity of pest infection or colonization. Therefore, in substrate-based studies, the host takes on a more passive role in the disease triangle.

Studies that quantify the effects of the pest on host physiology can indicate how the host plays a more active role in the disease triangle. For example, insect feeding can result in down-regulated photosynthesis, conductance, or cause uncontrollable water loss in the host plant. These effects are typically captured at the leaf –level, where leaf response may depend on the different types of insect feeding guilds (Welter 1989), veinal vs. interveinal feeding (Sack et al. 2003, Aldea et al. 2005), or diffuse vs. concentrated feeding patterns (Zangerl et al. 2002). The effects of leaf feeding on physiology often extend beyond the damaged area on the leaf (Zangerl et al. 2002). These leaf-level studies can have implications for how the plant is coping or compensating for pest damage at the whole-plant level.

The most sophisticated studies of the role of host physiology in the disease triangle assess the host plant as an integrated, coordinated system. Few studies have considered the coordinated functions that the host uses to maintain homeostasis following pest damage; however, these studies can contribute the most to the perception of the host as an active part of the disease triangle. This is demonstrated in studies that quantify changes in whole-plant transpiration following herbivory (Meinzer and Grantz 1990, Pataki et al. 1998, Tausend et al. 2000). When whole-plant physiology is combined with leaf –level measures, coordinated adjustments in stomatal conductance and water potential can typically be detected following defoliation (Meinzer and Grantz 1990,

Tausend et al. 2000). Studies conducted at these higher organizational levels are able to capture integrated, whole plant responses to pest damage (Bucci et al. 2004).

APPROACH

The above outlined perspectives of the host, ranging from a passive substrate to an integrated, coordinated organism, can all be valuable for characterizing the role of the host in the interplay between the components of the disease triangle. Therefore, the chapters of this dissertation include all of the perspectives of the host. The first chapter includes the most passive perspective of the host, and considers host predisposition and susceptibility to a stem disease. In this first chapter I quantify host response to disease only in terms of the extent of stem disease development, and focus on the external factors affecting disease development. In the second chapter, I examine a more active perspective of the host by quantifying the physiological effects of stem disease damage at the leaf and whole plant-levels. In the second chapter I also identify mechanisms that the host uses to cope with and compensate for stem disease damage. In the third chapter I focus on the host as an integrated, coordinated organism, and consider the capacity of the host to compensate for, and actively respond to, foliar pest damage.

Study system

Located at the northern edge of temperate pest ranges, the boreal forest is predicted to be one of the most vulnerable regions to climate-related changes in plant-pest dynamics (Netherer and Schopf 2010). High-latitude warming is expected to release pests from previously restricting conditions and accelerate rates of pest development,

reproduction, and movement (Netherer and Schopf 2010, Woods et al. 2005). Warming in northern regions is also expected to be stressful for plant hosts. High-latitude climate changes often operate at a faster pace than the scale at which plants are able to migrate or adapt to the altered climate (Jump and Penuelas 2005, Garrett et al. 2006). This may push plants beyond the physiological limits of their current ranges (Garrett et al. 2006) and result in long-term stress. Therefore, high-latitude plants could experience increased vulnerability and susceptibility to disease (Coakley et al. 1999, Harvell et al. 2002) and a reduction in their ability to resist and recover from disease outbreaks (Jump and Penuelas 2005).

It is likely that climate-related changes in disease dynamics are already underway in the boreal region. Long periods of warmth and dryness over the last several decades have caused soil water deficits and accelerated evapotranspiration (Barber et al. 2000, Oechel 2000), subjecting multiple boreal forest species to temperature-induced drought stress, reduced growth, and increased vulnerability to disease (Brandt et al. 2003, Hogg et al. 2008, Nossov 2008).Climate-related stressors are thought to be associated with outbreaks of insects or disease in several boreal species, including aspen (*Populus tremuloides*) (Brandt et al. 2003, Hogg et al. 2008), white spruce (*Picea glauca*) (Juday et al. 2005), and thinleaf alder (*Alnus tenuifolia*) (Nossov 2008, Ruess et al. 2009). Studies based on long-term disease and climate records have confirmed that the effects of warming on disease and insect dynamics have already materialized in high-latitude forests of British Columbia and Fennoscandia (Woods et al. 2005, Kurtz et al. 2008, Jepsen et al. 2008).

I focused on a boreal shrub in Alaska, *Alnus viridis* subsp. *fruticosa* (Rupr.) Nym., (synonym= *A. crispa*); hereafter, *A. fruticosa*. This shrub is currently a host to several pest species that are either already at epidemic status or have recently achieved epidemic status on other *Alnus* species in Alaska. One of these pest species is the fungal canker pathogen *Valsa melanodiscus* (anamorph=*Cytospora umbrina*), a characteristically facultative pathogen that can cause extensive canker damage to hosts weakened by a variety of stressors (Adams et al. 2005). The canker disease is characterized by slightly sunken stem cankers that extend laterally along the stem, resulting in girdling and dieback (Stanosz et al. 2008, Worrall et al. 2010). Since 2004, this canker disease has been at epidemic levels on *Alnus tenuifolia* throughout Alaska (USDA 2010). The canker pathogens associated with this disease are more frequently found in the asexual state (the *Cytospora* anamorph) (Adams et al. 2005). Therefore, throughout this dissertation I discuss the general attributes of this pathogen as a *Cytospora* species. I conduct several inoculation experiments using specific isolates of this canker pathogen in the sexual state (*Valsa melanodiscus*).When I describe the specifics of these experiments, I refer to the canker pathogen as *V. melanodiscus*.

During this study, biotic damage from several Lepidoptera and Hymenoptera larvae gave me the opportunity to evaluate the effects of a completely different functional type of damage on *A. fruticosa*. In June 2005, *A. fruticosa* experienced an early season foliar chewing event that caused more damage than *A. fruticosa* typically experiences over the course of an entire growing season (~15%) (Mulder et al. 2008). Following this insect chewing event, I evaluated the physiological effects of this biotic damage on *A.*

fruticosa. Across Alaska, *Alnus* species have recently experienced higher levels of foliar damage from invasive insects. *S*everal invasive sawfly species have caused extensive defoliation on *Alnus* spp. throughout the state (USDA 2009), and a new invasive green alder sawfly (*Monsoma pulveratum*), which is native to Europe and North Africa was recently identified in Alaska (USDA 2010). Documentation of *M. pulveratum* in Alaska is the first record of this insect pest in the United States (USDA 2010).

The geographical extent, abundance, and growth of *A. fruticosa* in dense, relatively uniform stands qualifies this host to support a virulent pest that could cause widespread damage (Lovett et al. 2006). *A. fruticosa* comprises a large component of Alaska's vegetation and is a dominant shrub at latitudinal and altitudinal treeline (Mitchell and Ruess 2009). It is persistent throughout succession and ubiquitous throughout all stand types in the interior Alaska. The density and prevalence of *A. fruticosa* in many areas provides an extensive supply of dense host material for intercepting and transferring inoculum or insect pests (Burdon and Chilvers 1982). Furthermore, as a nitrogen-fixing shrub, *A. fruticosa* provides a significant input of nitrogen to the boreal system (Mitchell and Ruess 2009) and widespread damage to this shrub could translate to long-term, ecosystem-level consequences (USDA 2010).

Since the range and severity of insect and disease outbreaks is already materializing at northern latitude forests (Woods et al. 2005, Kurtz et al. 2008, Jepsen et al. 2008), can we also expect *A. fruticosa* to experience higher amounts of disease damage under Alaska's warming climate? Although this appears to be the current trend in

northern forests, the level of specificity and complexity that is inherent in plant-pest interactions makes it difficult to make predictions across plant-pest systems (Roy et al. 2004, Wiedermann et al. 2007), even for a single host species (Adams et al. 2005). This complexity fuels a level of uncertainty in the predictions of plant disease dynamics that can only be addressed by increased knowledge of specific plant-pest interactions (Runion 2003). Some predictions for specific plant-pest systems rely on the environmental requirements of plants and their pests to predict the outcome of disease, but this approach has been labeled "attractive" but "simplistic" (Coakley et al. 1999). Therefore, characterizing the complexities of the plant-pathogen-environment interaction at a detailed level can capture important mechanisms and inform dynamics that may be operating at broader scales (Coakley et al. 1999).

Outline of thesis

The overall aim of my work was to evaluate the response of *A. fruticosa* to two types of pest damage against the backdrop of a warming boreal forest. The main body of this dissertation is arranged according to the perspective of the host as a passive substrate (Ch. 2, 3) to an integrated organism (Ch. 3, 4) (Fig.1.2).

Chapter 2 and 3 include inoculation trials on *A. fruticosa* using the fungal pathogen, *Valsa melanodiscus*. These chapters address whether host predisposition and susceptibility to disease were related to stressful conditions in field (Ch. 2) and in the greenhouse (Ch.3). In chapters 3 and 4, the host takes on a more active role in the disease triangle. Chapter 3 focuses on the physiological effects of the stem canker disease

on *A. fruticosa* and coping mechanisms in the host. Chapter 4 evaluates the physiological effects of foliar herbivore damage and compensatory water loss following leaf area damage (Fig. 1.2).

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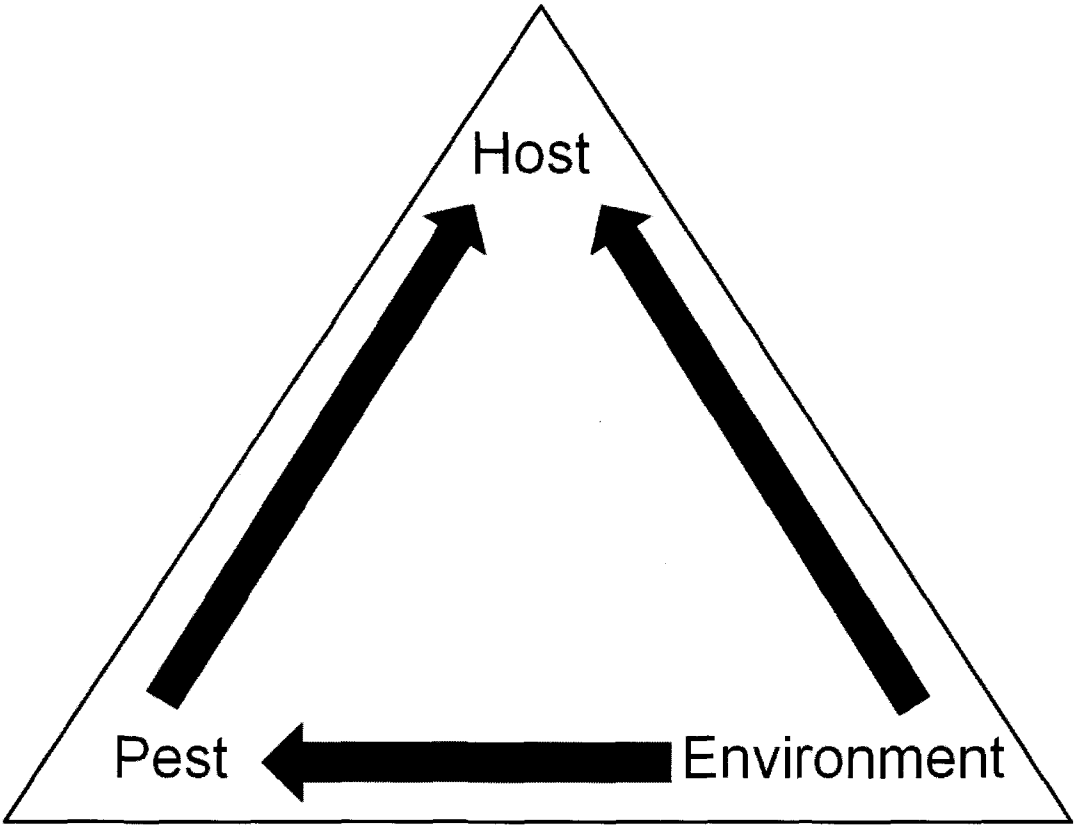

Figure 1.1 The disease triangle. Disease outcome is a function of the interplay of the host, the pest, and the environment.

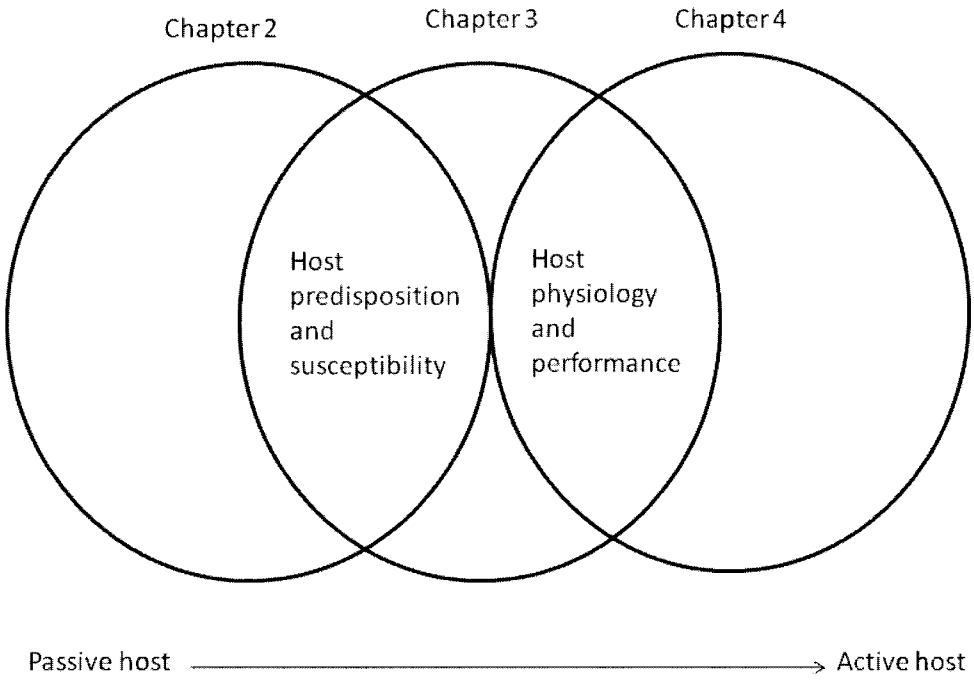

Figure 1.2 The relationship between the dissertation chapters. Chapter 2 does not consider any active responses of the host to disease damage and therefore has a "passive" perspective on the host. Chapter 3 focuses on leaf-level responses to disease damage and Chapter 4 focuses on ramet-level responses to herbivore damage. These chapters evaluate host mechanisms for coping with or compensating for disease damage.

CHAPTER 2

RESPONSE OF *ALNUS FRUTICOSA* TO INOCULATION WITH *VALSA MELANODISCUS* IN ROADSIDE AND FORESTED ENVIRONMENTS[1]

ABSTRACT

Valsa melanodiscus (anamorph *Cytospora umbrina*) has been associated with cankers and mortality of alder (*Alnus*) in Alaska. There is a wide range of virulence and aggressiveness among *Cytospora* species, and only responses to inoculation can be used to determine if a *Cytospora* species is able to incite disease on a particular host plant. We conducted a field inoculation study to determine whether *V. melanodiscus* isolated from *Alnus tenuifolia* is capable of inciting cankers on *Alnus fruticosa*. We also evaluated the potential for location in roadside versus forested environments to affect disease predisposition and susceptibility. In July 2008, we inoculated stems by placing a colonized agar plug over a wound that exposed the inner bark and sapwood. Inoculated stems developed sunken, brown elliptical cankers and wounded control stems produced abundant callus and wound closure. *A. fruticosa* located along a south-facing roadside were the most susceptible to disease, suggesting that the combination of high solar radiation, high air temperature, and poor roadside soil conditions could be related to higher disease severity.

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INTRODUCTION

An epidemic of disease on *Alnus incana* ssp. *tenuifolia* (hereafter, *Alnus tenuifolia*), has been documented throughout the Rocky Mountain West since the late 1980s (Worrall 2009) and has recently been documented in Alaska (USDA 2009, 2010). Alaskan surveys indicate that thousands of hectares of dieback and mortality of *A. tenuifolia*, an early successional shrub that colonizes broad floodplains and riparian areas in Alaska (USDA 2010), are related to the occurrence of Cytospora canker disease. Cankers are most severe on *A. tenuifolia*, but the same disease symptoms and signs have also been observed on *Alnus fruticosa*, an upland shrub that is persistent throughout succession (USDA 2009). Both *Alnus* species are symbiotic nitrogen fixers and are responsible for high N-inputs in Alaskan ecosystems (Uliassi and Ruess 2002, Mitchell and Ruess 2009).

The Cytospora canker disease of alder is characterized by slightly sunken stem cankers that extend laterally along the stem, resulting in girdling and dieback (Stanosz et al. 2008, Worrall et al. 2010). The disease has been frequently associated with *Valsa melanodiscus* (anamorph=*Cytospora umbrina*) (Adams 2008), which typically colonizes and kills the inner bark (bark periderm) and vascular tissues (Adams et al. 2005). Asexual *Cytospora* fruiting bodies consist of clusters of pycnidial conidiomata or conidiomata that contain labyrinthine chambers (Adams et al. 2006). The most obvious signs of Cytospora canker are abundantly produced conidiomata on canker faces, and, in moist conditions, exuding conidia in a gelatinous matrix of orange or cream colored tendrils (cirrhi)

(Adams et al. 2005, Adams et al. 2006). However, identification of a particular *Cytospora* species based on the fruiting bodies is difficult because the morphology of the anamorph and teleomorph can differ according to characteristics of the host bark and cambium (Adams et al. 2005).

Although the Alaskan canker disease epidemic has been associated with *Cytospora umbrina*, some *Cytospora* species are not characteristically primary inciting agents of disease. Despite their prevalence as canker fungi on over 85 species of woody hosts (Sinclair et al. 1987), most *Cytospora* species have not been tested for pathogenicity and the parasitic nature of some members of the genus is unclear (Adams et al. 2005). In general, Cytospora fungi are thought to inhabit asymptomatic living plants (Chapela 1989, Adams et al. 2005). Some *Cytospora* species may be strictly saprophytic on dying trees (Christensen 1940), and because fruiting bodies are often found in dead twigs and damaged bark, it can be unclear whether the fungus is a pathogen or a saprophyte (Adams et al. 2005). However, *Cytospora* fungi can be facultative parasites and cause extensive damage when plants are weakened by wounding (Christensen 1940, Kamiri and Laemmlen 1981, Filip et al. 1992) or under a range of other biotic stressors (Adams et al. 2005). Furthermore, *Cytospora* species can be very destructive pathogens on certain groups of plants, including cultivated species of *Prunus* (Adams et al. 2006) and *Populus* (Kepley and Jacobi 2000).

Given the wide range of virulence and aggressiveness among *Cytospora* species, only responses to inoculation can be used to determine if a *Cytospora* species is able to

incite and cause disease on a particular host plant. Following the Cytospora canker epidemic in Alaska, *V. melanodiscus* was confirmed to be pathogenic on *A. tenuifolia* (Stanosz et al. 2008). However, whether *A. fruticosa* is included among hosts of this pathogen has yet to be confirmed. Therefore, the first goal of this study was to conduct an inoculation trial to determine whether the same pathogen (*V. melanodiscus*) associated with canker disease and extensive mortality in *A. tenuifolia* can also incite disease in *A. fruticosa*.

There is circumstantial evidence that a summer drought in 2004 was a principal factor in the development of the Cytospora canker epidemic in Alaska (Ruess et al. 2009). The rapid development of canker disease coincided with one of the hottest, driest summers on record in 2004 (Ruess et al. 2009) and the stressful summer conditions were reflected in suppressed radial growth of *A. tenuifolia* (Nossov 2008). Although Cytospora canker diseases are not always associated with drought (Kepley and Jacobi 2000), water stress has long been implicated as a factor that can increase host predisposition or susceptibility to Cytospora diseases (Adams et al. 2005). This was first explored using shoot cuttings of *Populus* (Bloomberg 1962, Bier 1953) and has also been observed in potted *Picea pungens* (Kamiri and Laemmlen 1981) and *Populus* sp. (Guyon et al. 1996). Host predisposition and susceptibility to Cytospora canker disease has also been attributed to other abiotic stressors, including: injury to buds and dormant twigs by low temperatures (Helton 1961), frost injury (Reich and van der Kamp 1993), metal accumulation in soils (Abebe and Hart 1990), and fire injury (Dearness and Hansbrough 1934).

Our second goal was to address the potential for a combination of stressors to affect the predisposition and susceptibility of *A. fruticosa* to *V. melanodiscus*. We conducted a component of the inoculation trials along roadsides, which are typically colonized by *A. fruticosa* and present a reliable combination of stressors. Poor soil conditions along roadsides, such as high bulk density, low organic matter, and high soil compaction (Karim and Mallik 2008), are likely to limit water availability to roadside plants. This has been problematic in urban environments, where high soil compaction has resulted in a level of water-limitation that predisposes trees to the Cytospora canker diseae (Adams et al. 2005). Therefore, we expected that inoculated roadside alders would be more vulnerable to water stress and susceptible to the canker disease compared to similarly inoculated alders on wooded transects.

METHODS

Site selection

This study took place in the interior of Alaska, which has a cold, semi-arid climate. We conducted our study at sites that were part of a larger, long-term study (2001-2008), which evaluated cumulative herbivore and pathogen damage on *A. fruticosa* across a local climate gradient (Mulder et al. 2008). Three replicate sites (BNZ-South, East, West) at the Bonanza Creek Experimental Forest LTER (64°44′80″N, 148°19′20″W), 20 km south of Fairbanks were located in mixed stands of predominantly white spruce (*Picea glauca* (Moench) Voss), birch (*Betula neoalaskana*) or aspen (*Populus tremuloides* Michx.). These upland, secondary successional stands had similar stand

characteristics: dense canopy cover (76-79%), low coverage by mosses and lichens (0-2% ground cover), and low litter accumulation (0-5.0 cm depth). Although chosen for similarity of forest type and stand characteristics, the sites differed in elevation and aspect (Table 2.1).

Roadside conditions

The Bonanza Creek Experimental Forest Road is a dirt road that is lightly maintained and approximately 5 m wide. Roadside alders were growing on the bank of a road cut, where the soil is frequently disturbed and erodes with heavy rains. The organic layer of soil had been stripped away, leaving the mineral layer exposed, with pockets of light organic, eroded debris in areas of the roadcut. The roadside was heavily colonized by *A. fruticosa*, but other understory shrubs (*Salix* spp.), several herbs (e.g. *Chamerion angustifolium*), and grasses were also present. In contrast to the roadside, alder density was lower along the wooded transect and the overstory canopy was relatively closed by white spruce (*Picea glauca*), birch (*Betula papyrifera*) or aspen (*Populus tremuloides*). The wooded transects were also characterized by a duff layer several cm deep, a well accumulated organic soil layer, and patches of moss and grass on the forest floor. One of the most common understory species in these stands is bog cranberry (*Vaccinium vitis-idaea*).

Environmental conditions

Air temperature and relative humidity have been monitored at the three Bonanza Creek sites since 2004. Soil moisture, canopy density, moss depth, litter accumulation,

and percent cover of different vegetation layers were also characterized in 2004. To quantify any additional environmental differences between the roadside and wooded transects, data loggers (HOBO H8 Pro Series, Onset Comp. Corp., Bourne, MA.) recording air temperature and relative humidity at 30 minute intervals were mounted at 1.2 m and launched in August 2010. Radiation sensors (LI-190 SA Quantum Sensor, LI-COR, Lincoln, Nebraska) that were connected to data loggers (LI-1400 data logger, LI-COR, Lincoln, Nebraska) were placed in pairs at each site, with one sensor on the roadside transect and one sensor placed in the woods. Volumetric soil moisture sensors (SM200 soil moisture sensor, GP1 Data Logger, Delta-T Devices Ltd., England) were also placed in pairs at each site. Soil moisture sensors were installed at the sites using surface installation, which measured the top 5 cm of the soil. Soil temperature at 10 cm depth (digital MULTI-thermometer, Infrared Thermometers.net) was also measured in August 2010. Data were downloaded from the loggers in October. This ~ 3 month measurement period captured several weeks of sunny, high temperature weather during late summer and early fall.

Ramet selection

Alders were inoculated between 30 June and 5 July 2008. At each site, we established a wooded transect and a parallel roadside transect that bordered the Bonanza Creek Experimental Forest Road. The wooded transect was at least 25 meters from the roadside and began adjacent to the long-term study sites (Mulder et al. 2008). Along each transect, we selected 19 ramets that were within a diameter range of 15-28 mm. We

chose ramets at 3-5 meter intervals to ensure that we were selecting genetically distinct individuals. Ramets were randomly assigned to a treatment (control, Isolate 1, Isolate 2). On each transect, eight ramets were inoculated with Isolate 1, eight ramets were inoculated with Isolate 2, and three ramets served as controls. For the three separate sites at Bonanza Creek, we inoculated 16 ramets per transect and 32 ramets per site, for a total of 96 inoculated ramets plus 18 control ramets.

Fungal isolates

Two *V. melanodiscus* isolates were used to produce inoculum: Jim's Landing 2 (06-08) and Helmaur 1 (06-12) (Adams 2008), hereafter referred to as Isolate 1 and Isolate 2. Both were obtained from cankers on *A. tenuifolia* in Alaska and were collected and identified by Gerard Adams (Adams 2008). These isolates were chosen for this study because they are of proven pathogenicity on *A. tenuifolia* and were associated with a canker disease outbreak (Stanosz et al. 2008).The same isolates have also been used in greenhouse inoculation studies with *A. fruticosa* (Rohrs-Richey et al. 2010) and in greenhouse and field inoculation studies with *Alnus tenuifolia* (Stanosz et al. unpublished data). Cultures were maintained on potato dextrose agar (PDA) (Fisher Scientific, Houston, TX., USA) at 17°C.

Inoculation procedure

Plugs of inoculum (10 mm x 5 mm) were cut from the actively growing margins of *V. melanodiscus* cultures on PDA. The inoculation site (on the stem 50 cm above the soil surface) was wiped with a cloth wetted with tapwater and then with a cloth wetted

with 95% EtOH. A scalpel was used to make a wound (10 mm x 5 mm) exposing the sapwood (Rohrs-Richey et al. 2010, Stanosz et al. 2008). An inoculum plug was positioned on the wound with mycelium facing the sapwood and secured with Parafilm (American National Can, Greenwich, CT., USA). Control stems were similarly treated, except a sterile PDA plug was used. The Parafilm was removed 4 weeks after inoculation.

Assessing external and internal symptoms

All stems were evaluated in the field approximately 2 months after inoculation (5 September 2008). On this date, we measured the length of externally visible bark necrosis associated with wounding and inoculation. Stems were harvested 1 year later (August 2009) and evaluated for external and internal symptoms. The dimensions of externally visible necrotic bark were measured. The dimensions (length and width) of callus were also measured on each side of the inoculation point, as well as the dimensions of dead, exposed sapwood. Percentage girdle was calculated based on stem diameter measurements 50 mm adjacent and proximal to the inoculation point (beyond the length of necrosis) and measurements within the necrotic regions. Other visible external disease symptoms were recorded, such as bark cracking. The internal canker dimensions were measured on the sapwood surface, which was exposed by shaving away the outer bark and underlying layers of periderm with a razor blade. On a subset of inoculated stems (n=22), we peeled back the bark and cut deeply into the sapwood to determine the length of discolored tissue extending from the margins of the internal parts of the canker.

Culturing from the inoculated stem

Segments (50-60 cm in length, centered on the inoculation point) were harvested during the last week of August 2009, placed in plastic bags, and refrigerated. Within several days of harvest, culturing of *V. melanodiscus* was attempted for each inoculated and control stem. The bark and callused tissue was peeled away, exposing the underlying sapwood. Four chips of wood from the distal margin of the canker (or distal to the wounding point on the control stems) were surface disinfested (95% EtOH rinse for 20 seconds) and placed on PDA. Cultures were then incubated in the dark at ambient laboratory temperature. Plates were checked daily for colony characteristics, including conidiomata, that are indicative of *V.melanodiscus*. Transfers were made from a subset of one-third of the positive cultures to re-isolate *V. melanodiscus* in pure culture.

Data analysis

Variation in external and internal disease symptoms was first explained by stem treatment (control or inoculated). For wounded and inoculated stems only, ANOVA was used to explain variation of internal and external disease symptoms according to site, transect, isolate, and interactions of the main effects. Normality of all variables was checked before entry into the designated model and transformations were made as necessary. All analyses were performed using SAS (SAS Inst. version 9, Cary, N.C.).

RESULTS

External disease symptoms

When stems were evaluated eight weeks after inoculation, only two stems had external disease symptoms. These stems had developed 1.0 cm and 0.7 cm long necrotic lesions that extended longitudinally from the point of inoculation. Both of the diseased stems were located along the roadside transect at BNZ6F. All other inoculated stems had only 1 mm or less bands of bark necrosis surrounding the inoculation point, a typical necrotic response to wounding that was visible on the control stems.

When the stems were harvested and evaluated for disease (in August 2009), more severe disease symptoms had developed on the inoculated stems. Beyond the length of the original wound, 36% of the stems had developed necrotic lesions ≥ 5 mm long and 19 % of the stems had developed lesions ≥ 10 mm long. Thirty-four percent of inoculated stems only had a typical wounding in response to inoculation (bark necrosis $\leq$ 1mm). Percentage girdle ranged from 4 to 15%, and none of the lesions girdled the stem entirely (Table 2.2).

Severe symptoms included sunken, brown cankers in an elliptical area around the inoculation point (Fig. 2.1). Conidiomata were not seen on the surface of the cankers. Discoloration was most visible on stems with smoother, lighter green bark but was less distinct on stems with rougher bark. Longitudinal bark cracks were also common, with 1 to 3 longitudinal cracks (29.67 $\pm$ 3.45 mm) typically present in the vicinity of cankers.

Regardless of the severity of disease symptoms, necrotic bark was surrounded, but not contained by, callus. The dimensions of callused tissue (16. 24 ± 2.89 mm long, 2.89 mm wide) were not large enough to close off the initial wound. At the inoculation site, the sapwood was dried out and exposed. The dimensions of exposed sapwood (13.38 ± 0.39 mm long, 6.13 ± 0.18 mm wide) were slightly greater than the dimensions of the initial stem wound (10 x 5 mm).

Lesions did not develop on the control stems, which had less than 1 mm wide band of necrotic tissue less than 1 mm wide associated with the initial wound. Control stems developed callus (8.41 ± 1.69 mm long) in response to wounding, which was half the length of the callus produced on inoculated stems (15.3 ± 0.63 mm long) ($F_{1,110}$=26.86, P<0.0001). The callused tissue on control stems extended in width (2.8 to 3.2 mm) on either sides of the initial wound. Callusing entirely closed the wound in 53% of the control stems.

Internal disease symptoms

In association with the externally visible lesions, *A. fruticosa* developed internal lesions. The mean dimensions of internal cankers (24.42 ± 0.82 mm x 7.05 ± 0.23 mm) were much larger than the dimensions of the associated external cankers (6.53 ± 0.75 mm x 2.69 ± 0.19 mm). It was difficult to evaluate lesion extent at consistent depth within host tissue for all stem samples. Therefore, while internal lesions were consistently greater than external lesions, reported dimensions of internal lesions are only an estimate of lesion extent. Deep cuts into the xylem of a subset of inoculated stems revealed brown

discoloration that extended longitudinally from the point of inoculation and also to the pith. For these stems, the length of discolored sapwood (249. 77 ± 28.22 mm) extended far beyond the external margins of the length of externally visible canker (4.45 ± 0.88 mm) (Fig. 2.2).

V. melanodiscus was cultured from 91.5 % of the inoculated stems. Development of a pink to red coloration of the agar was the quickest indicator of *V. melandodiscus* in culture. This occurred within 1 week and the agar became darker red as the colony expanded. Twenty-five percent of the positive cultures developed pink-colored agar in 3-4 days. Within several weeks, the colonies produced conidiomata. Pure cultures of *V. melanodiscus* were obtained from all of positive cultures from which subcultures were made. Cultures of fungi obtained from control stems did not have any indications of presence of *V. melanodiscus*.

Environmental conditions on roadside vs. wooded transects

The roadsides at BNZ-South had the highest daily maximum temperatures and the greatest daily temperature fluctuations. During the three month measurement period, BNZ-South roadsides had the highest maximum air temperature (25.56 °C), while the maximum daily air temperature at other roadside sites did not exceed 22.09°C (BNZ-East) and 22.47°C (BNZ-West). The BNZ-South was also the only site where roadside air temperature reached higher maximum temperature than along the wooded transect (22.86°C). Air temperatures between the road and wooded transects were similar at BNZ- East and West. During the three month measurement period, the BNZ-South

roadside had a lower minimum temperature (-12.28˚C) than the roadsides at BNZ-East (-8.38˚C) and BNZ-West (-11.13˚C).

The BNZ-South roadsides also received higher daily levels of solar radiation (800-1000 μmol m^{-2} s^{-1}) compared to the other roadside sites (400-500 μmol m^{-2} s^{-1}). The wooded transects consistently had lower levels of solar radiation, with the lowest levels at BNZ-West where daily highs typically did not exceed 200 μmol m^{-2} s^{-1}. The high light, high temperature conditions along the BNZ-South roadside were also accompanied by low soil moisture. Volumetric soil moisture along the BNZ-South roadside remained between 11 to 13% for most of the measurement period, while soil moisture along the wooded transect varied from 16 to 30%. Following a rain event, soil moisture increased to 38% along the wooded transect but only 17% along the roadside.

Effect of site and roadside

Canker length was explained by site-transect interactions. At BNZ -South and BNZ -West, the alders along the roadside transects developed longer cankers than alders along wooded transects (Table 2,3). Canker development was greatest along the BNZ-South roadside transects (12.06 ± 2.20 mm long). Canker length was also explained by isolate-site interactions. Longer cankers developed in response to inoculation with Isolate 1, but only at the BNZ -South and BNZ-West sites (Table 2.2, 2.3).

In addition to longer cankers at BNZ-South site, alders at the BNZ -South site also had the greatest incidence (47%) of longitudinal bark cracks, while the incidence of alders with cracked bark was lower (26%) at other sites.

Percentage girdle was only explained by roadside versus wooded environment. Alders along the roadside transects had higher percent girdle (8.55 to 14.77%) than the wooded transects (4.17 to 8.96%) (Table 2.2, 2.3). The length of exposed sapwood and the length of callus tissue were not explained by site, transect, or isolate type.

DISCUSSION

Objective 1: response to inoculation

This is the first field study to confirm that *V. melanodiscus* is capable of inciting cankers on *A. fruticosa*. The external morphology of cankers on *A. fruticosa* was consistent with the response of *A. tenuifolia* to experimental infection with *V. melanodiscus* (Stanosz et al. 2008). In association with cankers, we also observed longitudinal bark cracking, which is symptomatic of fungal growth (mycelia spread) killing the bark in adjacent regions of the stem (Schreiner 1931). As fungal colonization advances in host tissues, longitudinal cracks can be formed by intercellular hyphal wedges that grow through the host periderm and crack the outer bark (Biggs et al. 1983).

Hyphal colonization of the xylem can also induce sapwood discoloration (Biggs et al. 1983), which we observed in *A. fruticosa*. In hardwoods, sapwood discoloration is a common response to colonization by *Cytospora* fungi (Adams et al. 2005), which can be isolated from regions of the discolored wood (Filip et al. 1992). The extent of discoloration is typically correlated with the size of the external canker; however, as we observed, discoloration often continues beyond the length of the canker margin (Rohrs-Richey et al. 2010) and can extend to the pith. Whether *V. melanodiscus* is present

throughout discolored tissues of *A. fruticosa* is not known, and could be the subject of future studies. In *A. tenuifolia*, however, *V. melanodiscus* has been isolated from the xylem and phloem tissues as far as 5 cm from visible canker margins, but it has been more commonly isolated from the surface of the bark (Worrall et al. 2010).

Unlike other experimental studies of Cytospora canker pathogens, *A. fruticosa* did not readily produce apparent disease symptoms in response to inoculation until more than two months following inoculation. This suggests that *A. fruticosa* had greater resistance to extensive pathogen colonization at the time of inoculation. We inoculated alders during the first week of July, when alders typically approach peak rates of nitrogen fixation and plant growth (Mitchell and Ruess, 2009). In correspondence with peak growth rates, the plant might have been able to maintain resistance mechanisms that prevented canker advance, including suberin and lignin production for mechanical barriers (Bloomberg 1962, Bloomberg and Farris 1963), non-specific wound healing (necrophylactic periderms and non-suberized impervious tissue) (Maxwell et al. 1997), and synthesis of secondary metabolites (McPartland and Schoeneweiss 1984, Boyer 1995). Previous studies with Cytospora pathogens report that in places with long, cold winters, maximum canker expansion can be achieved when trees are inoculated in the autumn (September-October) and tree defense mechanisms are compromised by seasonal dormancy (Adams et al. 2005).

Objective 2: The effect of roadsides on predisposition and susceptibility

V. melanodiscus was consistently cultured from inoculated stems regardless of host location on roadside or wooded transects, suggesting that stressful conditions were not prerequisite for disease development in this experiment. Field and greenhouse inoculation studies of Alaskan hosts *A. tenuifolia* and *A. fruticosa* also indicated that a period of host stress was not required for successful infection with *V. melanodiscus* (Stanosz et al. 2008, Rohrs-Richey et al. 2010). These studies contradict reports that environmental stresses are required for host predisposition to facultative canker pathogens (Schoeneweiss 1975, Guyon et al. 1996).

Although roadside stressors did not facilitate infection of *A. fruticosa*, these stressors did affect disease development. Across all sites, roadside alders had greater percentage girdle than alders along wooded transects. Also, roadside conditions at both BNZ-South and BNZ-West resulted in larger external cankers. This confirms that conditions along roadside locations at Bonanza Creek can increase the susceptibility to the canker disease.

Disease severity, indicated by longer external cankers and higher percentage girdle, was the greatest along the roadside at BNZ-South. Depending on the isolate type, alders at BNZ-South developed cankers that were 50-400% longer than alders on the wooded transect. Percentage girdle was also 61% greater on the roadside alders compared to the wooded alders (Table 2). This suggests that the combination of roadside conditions with a south-facing aspect can increase disease susceptibility. Higher disease severity

along the BNZ-South roadside was likely related to plant water stress. Compared to the other sites, the roadside environment at BNZ-South had the highest air temperatures and received the highest solar radiation, which drove greater evaporative demand along the roadside. This was reflected in low roadside soil moisture and likely resulted in plant water stress. Experimental tests have also confirmed that water stress can increase disease susceptibility of *A. fruticosa* to *V. melanodiscus* (Rohrs-Richey et al. 2010).

At BNZ-West, isolate type explained more variation in external canker length than transect location. Cankers associated with Isolate 1 were up to 4 times longer than cankers associated with Isolate 2 (Table 2). The seasonal climate at BNZ-West was more humid, with lower air temperature and less solar radiation than the other sites (Table 1). This air climate was likely related to the lower elevation of the BNZ-West site (Table 1). In the interior of Alaska, cold air sinks to low elevations and valley bottoms, resulting in characteristically cooler environments. The cooler, more humid environment at BNZ-West may have been more suitable for the expansion of Isolate 1.

Isolate interactions

At BNZ-South and BNZ-West, cankers induced by Isolate 1 were longer, suggesting that Isolate 1 had the potential to cause more damage in roadside environments. Currently, the variation among isolates of the fungal pathogen is not known, and the variation in host response to infection and colonization is also not known. However, our results do suggest that there is variation in isolate aggressiveness. Since our study is limited in scope, comparing the aggressiveness of *Cytopora* isolates *in situ*

on *A. fruticosa* must be approached cautiously because of the potential for individual shrubs to respond to environmental stressors (Adams et al. 2005). For example, the wide genetic potential for adapting to environmental conditions in poplars and eucalyptus results in high variation in the susceptibility to Cytospora fungi (Adams et al. 2005). Therefore, confirming differences in the aggressiveness of *Cytospora* strains would require comparison of disease response in genetically similar hosts under environmentally similar conditions (Adams et al. 2005).

Implications for the two Alnus species

The isolates we used in this experiment were cultured from cankers on *A. tenuifolia*, and we used these isolates because they are proven to be pathogenic and virulent on *A. tenuifolia* (Stanosz et al. 2008). Compared to *A. fruticosa*, experimental disease development appears to be more immediate and severe on *A. tenuifolia*, which developed lesions that ranged in length from 10 mm to 600 mm in response to inoculation with the same isolates used in this study (Stanosz et al. 2008). Although these isolates were more aggressive on *A. tenuifolia*, there is little reason to believe that the pathogen is locally adapted to *A. tenuifolia*. Isolates of *V. melanodiscus* across the state of Alaska show a high level of genetic variation (Adams 2008), indicating that the disease epidemic on *A. tenuifolia* was not the result of a small group of highly aggressive genotypes that became dominant in the pathogen population. Local adaptation of the pathogen to *A. tenuifolia* is also not evidenced across the landscape. If an aggressive pathogen isolate were locally adapted to its host, the disease would be most severe on

spatially isolated or distinct populations of *A. tenuifolia*. However, the vast extent of the canker disease outbreak on *A. tenuifolia* suggests that large-scale processes contributed to state-wide disease-related mortality.

Large-scale disease susceptibility in *A. tenuifolia* may be related to Alaska's changing climate, which has been warming over the last several decades (Barber et al. 2000, Oechel et al. 2000). Dendroecological studies indicate that *A. tenuifolia* has reduced growth rates under warm, dry conditions and circumstantial evidence suggests that these conditions contributed to the disease outbreak. The canker disease outbreak on *A. tenuifolia* appeared to coincide with the summer of 2004, one of the hottest, driest summers on record in the interior of Alaska (Ruess et al. 2009). As a riparian species, *A. tenuifolia* is most vulnerable to drought stress early in the growing season, when river discharge (from glacially fed rivers) is well below peak levels (Nossov 2008). When disease reached outbreak levels during the summer of 2004, meteorological drought and low river levels had resulted in landscape-level drought stress and reduced radial growth in *A. tenuifolia* (Nossov 2008).

Experimental results from this study and a greenhouse study (Rohrs-Richey et al. 2010) indicate that warmer, drier conditions can also increase disease susceptibility for *A. fruticosa*. However, as an upland species, *A. fruticosa* may be more adapted to long periods of water limitation and less vulnerable to drought stress. *A. fruticosa* has been shown to cope with water limitation by regulating transpirational water loss and maintaining plant water balance below a critical threshold (Rohrs-Richey, Ch. 4). The

ability to regulate water loss has been related to drought tolerance in *Alnus* species (Schrader et al. 2005). The ability to tolerate drought has not been determined for *A. tenuifolia*; however, the restriction of *A. tenuifolia* to riparian areas suggests that the distribution of this species is limited by drought tolerance (Schrader et al. 2005).

As climate warming is projected to continue in Alaska, the Cytospora canker disease could play a role in shifting the abundance of *A. tenuifolia* compared to *A. fruticosa*. If temperature continues to warm in Alaska, drought stress and growth suppression could become more common in *A. tenuifolia* and continued disease-related mortality may weaken the competitive ability of *A. tenuifolia* in riparian areas (Nossov 2008). A warmer, drier climate may also be stressful for *A. fruticosa*. However, this species may be physiologically more equipped to tolerate warmer, drier conditions and therefore may not be as susceptible to the Cytospora canker disease under Alaska's future climate.

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Table 2.1 Environmental parameters at the Bonanza Creek sites. Air and soil climate data represent the ranges in mean monthly data from June, July, and August in 2005 or 2006. All sites had a narrow range of minimum winter temperatures (-27.35 to -29.31 °C from December to February).

	BNZ- South	BNZ – East	BNZ –West
Elevation (m)	360	355	220
Aspect (°)	135	85	250
Air climate			
Temperature (°C)	11.6 – 16.4	13.7 – 18.4	9.1 – 14.1
Relative humidity (%)	66.9 – 82.3	62.6 – 83.5	73.2 – 93.3
Solar radiation (μmol m^{-2} s^{-1})	51.3 – 107.3	66.5 – 106.8	61.8 – 64.3
Soil climate			
Moisture (%)	6.1 – 27.8	9.7 – 31.5	12.2-29.1
Temperature (°C)	8.3 – 9.6	7.3 – 12.2	7.6 – 10.3

Table 2.2 Canker length and percentage girdle. Means ± S.D. by site, transect, and isolate.

	Wooded transect	Roadside transect	Culture results
	Length (mm) (% girdle)	Length (mm) (% girdle)	# of stems positive/ # of stems tested
BNZ South			
Isolate 1	6.86 ± 1.92 (8.96 ± 1.05%)	13.63 ± 3.98 (14.77 ± 0.54%)	13/15
Isolate 2	2.50 ± 0.50 (6.22 ± 1.53%)	10.50 ± 2.04 (10.13 ± 1.95 %)	14/16
BNZ East			
Isolate 1	3.25 ± 0.31 (--)	3.75 ± 0.62 (8.59 ± 1.67 %)	16/16
Isolate 2	5.63 ± 2.14 (4.17 ± 4.16 %)	5.75 ± 1.72 (10.29 ± 2.16 %)	15/16
BNZ West			
Isolate 1	6.29 ± 0.92 (6.04 ± 1.73 %)	9.13 ± 2.57 (13.52 ± 3.18 %)	14/15
Isolate 2	2.63 ± 0.26 (5.60 ± 1.54 %)	2.29 ± 0.29 (8.55 ± 1.43 %)	13/15

Table 2.3 ANOVA results explaining variation in canker length and girdle. Denominator degrees of freedom (d.f.) is 65. NS is listed where the P-value >0.1.

		Canker length		% Girdle	
Source	d.f.	F-value	P-value	F-value	P-value
Site	2	4.47	0.014	0.85	NS
Transect	1	8.56	0.005	18.56	<.0001
Isolate	1	7.39	0.008	3.29	0.075
Site*transect	2	5.24	0.007	0.12	NS
Site*isolate	2	6.95	0.002	1.39	NS
Transect*isolate	1	0.21	NS	1.30	NS
Site *trans*iso	2	0.98	NS	0.11	NS
Model	11	4.68	<.0001	2.55	0.011

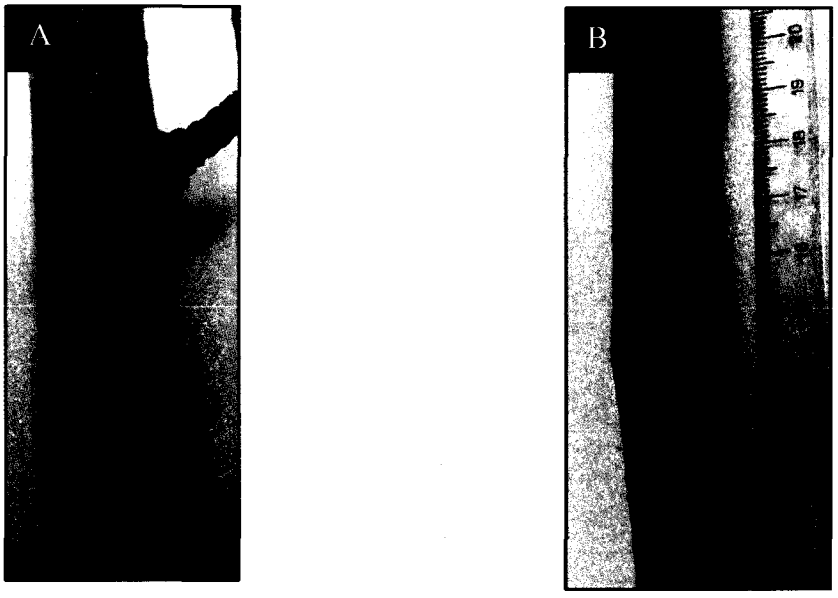

Figure 2.1 Example of canker development. Photos shown for cankers from the BNZ-South wooded (A) and roadside (B) transects.

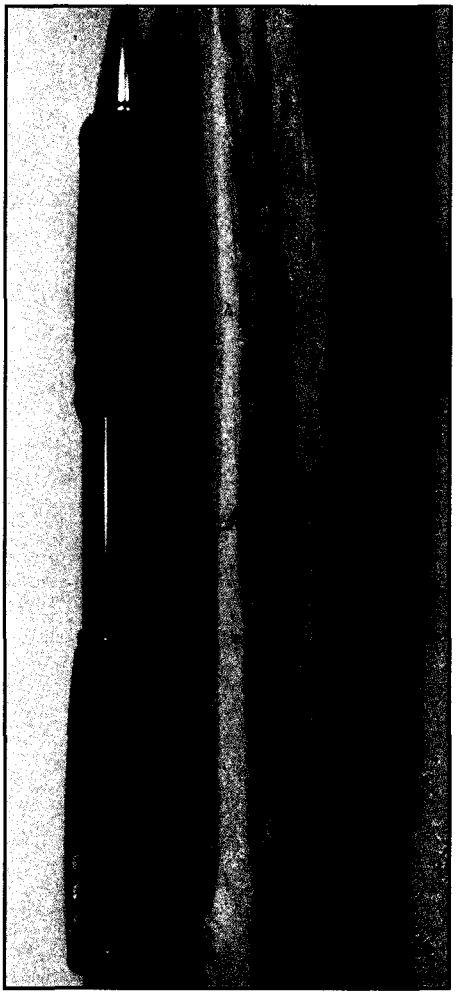

Figure 2.2 Discolored sapwood extending beyond the canker margins.

CHAPTER 3

PHYSIOLOGICAL PERFORMANCE OF AN ALASKAN SHRUB (*ALNUS FRUTICOSA*) IN RESPONSE TO DISEASE (*VALSA MELANODISCUS*) AND WATER STRESS[2]

ABSTRACT

- Following the decades-long warming and drying trend in Alaska, there is mounting evidence that temperature-induced drought stress is associated with disease outbreaks in the boreal forest. Recent evidence of this trend is an outbreak of Cytospora canker disease (fungal pathogen *Valsa melanodiscus* (anamorph =*Cytospora umbrina*)) on *Alnus* species.

- For *Alnus fruticosa*, we examined: 1) the effects of water stress on disease predisposition, and; 2) the effects of disease and water stress on host physiology. In two trials, we conducted a full-factorial experiment that crossed two levels of water stress with three types of inoculum (two isolates of *V. melanodiscus*, one control isolate).

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[2] Rohrs-Richey, J.K., Mulder, C.P.H., Winton, L. Stanosz, G.R. 2010. Physiological performance of an Alaskan shrub (*Alnus fruticosa*) in response to disease (*Valsa melanodiscus*) and water stress. New Phytologist. doi: 10.1111/j.1469-8137.2010.03472.x.

- Water stress was not required for disease predisposition. However, the effects of water stress and disease on host physiology were greatest near the peak phenological stage of the host and during hot, dry conditions. During this time, water stress and disease reduced light saturated photosynthesis (-30%), light saturation point (- 60%), and stomatal conductance (-40%).

- Our results depended on the timing of water stress and disease in relation to host phenology and the environment. These factors should not be overlooked in attempts to generalize predictions about the role of temperature-induced drought stress in this pathosystem.

INTRODUCTION

In the circumpolar north, there is considerable and compelling evidence that the climate to which plants are currently adapted is shifting (Jump & Penuelas, 2005; Sturm *et al.*, 2005; Tape *et al.*, 2006). High-latitude climate changes often operate at a faster pace than the scale at which plants are able to migrate or adapt to the altered climate (Jump & Penuelas, 2005; Garrett *et al.*, 2006). This may push plants beyond the physiological limits of their current ranges (Garrett *et al.*, 2006), resulting in long-term exposure to stresses such as high temperature or low precipitation. For example, long periods of warmth and dryness in the boreal forest over the last several decades have caused accelerated evapotranspiration and soil water deficits (Barber *et al.*, 2000; Oechel *et al.*, 2000) which in turn resulted in temperature-induced drought stress and reduced growth of multiple forest species (Brandt *et al.*, 2003; Juday *et al.*, 2005; Hogg *et al.*, 2008; Nossov, 2008). As a consequence of climate-related stressors, plants may not have the capacity to provide sufficient structural or biochemical defenses against diseases (Ayres, 1984; McPartland & Schoeneweiss, 1984; Boyer, 1995) or recover from disease damage (Ayres, 1984; Paul & Ayres, 1987; Ayres, 1991). For these reasons, it is generally predicted that plants will be more vulnerable to disease (Coakley *et al.*, 1999; Juday *et al.*, 2005) and experience higher disease incidence and severity with a shifting climate (Larsson, 1989; Mitchell *et al.*, 2003; Rodriguez *et al.*, 2004).

These predictions appear to be unfolding for Alaskan keystone shrubs, *Alnus* species, which are the dominant, symbiotic nitrogen-fixing shrubs in the boreal forest

(Uliassi & Ruess, 2002; Mitchell & Ruess, 2009). An outbreak of canker disease has caused significant dieback in *Alnus incana* ssp. *tenuifolia* (thin-leaf alder) and *Alnus fruticosa* (green alder), resulting in mortality and reduced nitrogen fixation throughout central and south-central Alaska (Ruess *et al.*, 2009). The disease is associated with the fungus *Valsa melanodiscus* (anamorph =*Cytospora umbrina*) and is characterized by long, girdling cankers (Adams, 2008; Stanosz *et al.*, 2008). The rapid development of this disease coincided with suppressed radial growth in *A. tenuifolia* (Nossov, 2008) during one of the hottest, driest summers on record in 2004 (Ruess *et al.*, 2009). Drought stress has been classically cited as a predisposing factor to Cytospora canker disease (Bier, 1953; Bloomberg, 1962; Bloomberg & Farris, 1963), and the drought event of 2004 prompted the working hypothesis that temperature-induced drought stress was a principal factor in the development of the disease epidemic (Ruess *et al.*, 2009).

The working hypothesis for canker disease in Alaska remains untested. Establishing causality between the summer conditions of 2004 and the canker disease epidemic requires long-term disease records in addition to crucial information about the three parts of the disease triangle: the host, pathogen, and environment (Harvell *et al.*, 2002; Woods *et al.*, 2005). The current canker epidemic on alder is the first on record for Alaska, so it is difficult to historically determine if this disease is related to the warming trend or is part of natural population cycles. Instead, we will have to rely heavily on information from the disease triangle to ascertain if the disease epidemic could be related to temperature-induced drought. Drought-related decline in host condition has been correlated to disease outbreaks in the boreal forest (Brandt *et al.*, 2003; Juday *et al.*,

2005; Hogg *et al.*, 2008), but there are no studies on the effects of canker disease and drought on the condition of *Alnus* species. For other hosts of Cytospora canker fungi, only static indicators of water stress, such as water potential (Guyon *et al.*, 1996; Kepley & Jacobi, 2000) or relative water status (Bloomberg, 1962; Tao *et al.*, 1984), have been used to gauge host condition. Our study measures host physiological response to canker disease and water stress using photosynthetic performance, stomatal conductance, sapflow, and water-use efficiency.

Our study is an experimental investigation of two types of disease-water stress relationships for *Alnus viridis* subsp. *fruticosa* (Rupr.) Nym., (synonym= *A. crispa*): 1) an effect of water-limitation on the susceptibility of hosts to disease (the predisposition concept), and 2) the combined effects of disease and water-limitation on host physiology (the multiple stress concept) (Desprez-Loustau *et al.*, 2006). The goal of the predisposition approach was to test the idea that the Cytospora canker pathogen will characteristically attack *A. fruticosa* hosts that have been weakened or compromised by water stress (Christensen, 1940; Manion, 1991), as observed on other hardwoods in natural systems and tested in experimental settings (Bier, 1953; Bloomberg, 1962; Bloomberg & Farris, 1963; Kamiri & Laemmlen, 1981; Guyon *et al.*, 1996; Kepley & Jacobi, 2000). The goal of the multiple stress approach was to evaluate the effects of simultaneous disease and water-limitation on the physiological performance of *A. fruticosa*, as one stressor is likely to exacerbate the effects of the other and reduce the capacity of the host to compensate or recover from disease (Ayres, 1984; Paul & Ayres, 1987; Ayres, 1991).

MATERIALS and METHODS

Plant material

In March 2005, *A. fruticosa* seeds were collected at nine sites within 50 km of the University of Alaska, Fairbanks, Alaska (64°51'28"N 147°51'23"W). Seeds from the cones of 36 plants were germinated in a soil media with a ratio of two parts peat, one part vermiculite, and one part coconut coir, and established seedlings were transplanted to 328 cm^3 "cone-tainers" (Stuewe and Sons, Tangent, OR., USA). After two years of growth, plants were transplanted into larger 983 cm^3 pots using the same peat-vermiculite-coconut soil media. Individual plants (genets) developed between one to four stems (ramets) and were pruned several times during the course of their growth. Five weeks before experimental treatments began (July, 2007), all ramets were pruned to a height of 200 mm. Five containers were placed with equal spacing in a rack, and the 34 racks were rotated weekly around the greenhouse benches.

Fungal isolates

Two *Valsa melanodiscus* isolates were used to produce inoculum: Jim's Landing 2 (06-08) and Helmaur 1 (06-12), hereafter referred to as Isolate 1 and Isolate 2. Both of these isolates were obtained from cankers on *Alnus tenuifolia* in Alaska and were collected and identified by Gerald Adams (Adams, 2008). Cultures were maintained on potato dextrose agar (Fisher Scientific, Houston, TX., USA) at 17 °C.

Experimental design

The experiment was conducted in two trials. Trial I began on July 13th 2007 and Trial II began approximately one month later, on August 23rd. Each trial was conducted as a completely randomized full-factorial design with two water treatment levels (well-watered or water-limited) crossed with three levels of inoculum type (Isolate 1, Isolate 2, or plain potato dextrose agar as a control inoculum), which resulted in six treatment combinations. There were 15 replicates (alders) per treatment combination and 90 plants per trial. Plants were randomly assigned to a water treatment level and then one ramet per plant was randomly assigned to an isolate type.

Greenhouse conditions

Greenhouse temperature was set in the range of 18-26°C with a photoperiod of 21 hr (maximum photoperiod for interior Alaska). Supplemental lighting from high pressure sodium and mercury lamps provided 300 μmol m^{-2} s^{-1} at bench height. Environmental conditions in the greenhouse zone (80 m^2) were recorded with the climate monitoring system (Hortimax, the Netherlands), including relative humidity, temperature, and light.

Inoculation

Plugs of inoculum (10 mm x 5 mm) bearing mycelium were cut from the active margins of 12-day old cultures of *V. melanodisus*. The inoculation site (on the stem 10 mm above the soil surface) was wiped with 95% EtOH, and a scalpel was used to make single wound (10 mm x 5 mm) exposing the sapwood. Inoculum plugs were positioned

on the wound with mycelium facing the sapwood and secured with Parafilm (American National Can, Greenwich, CT., USA), which was kept in place for two weeks. Stem wounds of this size and larger are naturally associated with snowshoe hare browsing, heavy snow-loading, or frost damage.

One ramet per plant was inoculated; if there were multiple ramets on the plant, the ramet to be inoculated was randomly selected. Necrotic lesions began developing beneath the Parafilm 1 week after inoculation, and the dimensions of developing cankers were measured at two week intervals for three months following inoculation. Disease incidence was recorded as positive if necrosis advanced more than 2 mm around the initial wound (typical necrotic response to control inoculum). Canker extent was estimated as an elliptical area based on length and width measurements. We measured internal colonization of the pathogen on a subset of 35 plants not used for physiological measurements. On this subset, the bark was peeled away to expose vascular tissue and the vertical extent of pathologically darkened tissue was measured. *V. melanodiscus* was re-cultured from all experimental cankers to confirm that the fungal pathogen was consistently associated with the disease symptoms. The cultures stained the agar reddish and often produced conidiomata (asexual fruiting structures).

Water treatments

Plants were watered by hand with reverse osmosis (RO) water. Water soluble fertilizer (Sunshine technigro 10-30-20, Sun gro Horticulture, Vancouver, Canada, combined with 20-10-20 and Sprint 330 iron chelate micronutrient) was applied once a

week in equal volumes (150 ml^3) to all plants. In both trials, the water limitation treatment began two weeks prior to inoculation and involved the application of low volumes of water for an average of four days followed by a short period (1-2 days) of no water. In contrast, well-watered alders were watered daily and generally received three to four times the water volume of water-limited alders. We adjusted the watering regime in accordance with plant growth and greenhouse conditions. For example, during warmer greenhouse conditions in July and August, the well-watered group received 450-600 ml^3 water while the water limited group received 150 ml^3. The level of water stress was carefully determined using physiological measurements and observing physical signs of water stress. Our objective was to maintain moderate water stress that would still enable leaves to respond to light curve and gas exchange measurements. We avoided high levels of water stress that resulted in full stomatal closure, wilting, excessive leaf shedding, or mortality.

Growth measures

Ramet height, leaf number, and ramet diameter were measured in each trial just before inoculation and eight weeks after inoculation. At the end of the experiment, in September, aboveground biomass was measured using dry weights of ramets and leaves from a subset of 80 randomly harvested plants. Specific leaf area ($cm^2\ g^{-1}$) for alders in each of the water treatments was also measured for at the end of the experiment using 15-20 leaf punches from leaves on a subset of 50 randomly selected plants.

Plant water status

Our priority was to maintain intact experimental plants and avoid the risk of additional infections from the combination of further wounding within a close vicinity of high inoculum loads. Therefore, we did not perform any destructive water status measurements on the experimental plants.

We used several types of physiological measurements as an index of plant water status. First, we took weekly measurements of stomatal conductance and transpiration rates (LICOR 6400, LICOR Biosciences, Lincoln, NE., USA) on plants in Trial I and II. Second, we measured monthly water potential (PMS pressure chamber, Albany, OR., USA) on ramets from a set of 20 plants that had been randomly selected for destructive measurements. Additionally, we continually monitored sapflow (Flow 32 Sapflow monitoring system, Dynamax, Inc., Houston, TX., USA) on ramets from a set of eight well-watered plants as a long-term indication of total plant water loss over the entire experiment. Physiological measurements were possible as the majority of ramets only developed sub-lethal cankers (necrotic lesions that did not girdle the entire stem).

Sapflow

Intact ramets on eight randomly selected plants from the well-watered treatment were fitted with small, external sapflow gauges (micro flow gauges SGA3, SGA5, Dynamax, Inc. Houston, TX., USA) and transpirational water loss was estimated by a heat balance method described by Baker & van Bavel (1987). Sapflow was only measured on ramets from well-watered plants because it was difficult to detect the heat

signal from low water flow in the water-limited treatment. Sapflow was monitored throughout the experiment on four ramets from the Isolate 1 treatment and three ramets that were not treated. The gauges and adjacent portions of the stem were wrapped with foam insulation and then reflective foil to minimize radiating heating of the stem. A gauge on one ramet was operated without power to the heater to be certain that the foil and foam insulation shielded the stem from external temperature fluctuations (Gutierrez *et al.*, 1994). For a two week period at the end of Trial II, we re-arranged the gauges so that sapflow could be measured below and above the stem canker. Four ramets were fitted with two sensors, and each sensor was attached to the stem adjacently to the upper or lower region of a canker. A data logger (Model CR10x, Campbell Scientific Corp., Logan, UT., USA) continuously recorded mass flow of sap and averages were logged every 15 minutes.

Light response curves

We measured light response curves (LRC) using a LI- 6400 (LICOR Biosciences, Lincoln, NE., USA). We used a split-plot design, where water treatment was applied at the whole-plot level (individual plant) and the inoculum treatment was applied at the subplot level (ramet). This split plot design was used for two groups of plants: a disease group and a no-disease group. For plants in the disease group, we tested the effects of disease on light response. Light response was measured on leaves from different ramets on the same plant: an untreated ramet (Control ramet) and a ramet wounded and treated with inoculum from Isolate 1 or 2 (Diseased ramet). In the disease group, LRC

measurements were made on three to four plants from each treatment combination that were selected based on the similarity of diameter, height, and leaf number of the paired ramets. The same split-plot design was used for plants in the no-disease group, which tested the effect of the inoculation procedure (wounding and agar application) on light response. Light response was measured on leaves from paired ramets on the same plant: an untreated ramet (Control ramet) and a ramet that was wounded and received an agar-only plug (Control inoculum). For each trial, we measured 4-6 alders in the no-disease group, which were also selected based on similar morphology of the paired ramets. Multivariate ANOVA confirmed that the small wound and agar plug did not affect light response, as all light response curve parameters were similar between the paired ramets from the no-disease group. Wounding only explained 6% of the variation in light response in August ($F_{3,14}$=0.096, P=0.438) and less than 1% of the variation in September ($F_{3,14}$=0.07, P=0.977). Therefore, we only report results that describe the differences between the paired ramets (control vs. diseased) in the disease group.

We measured light response curves on these plants in the beginning of August and September. The most recently-expanded leaf was used for the LRC measurements. A portion of the leaf was enclosed in a cuvette with an area of 100 mm^2, which was regulated for temperature, airflow, humidity, and irradiance. Leaves were measured between 11:30-15:30 hr each day. Automatically programmed light response curves were used starting with a high light level (2500 $\mu mol\ m^{-2}s\text{-}^{1}$), constant reference CO_2 (400 $\mu mol\ CO_2\ mol^{-1}$), a constant air flow (500 $\mu mol\ s^{-1}$), and set points for chamber humidity, and leaf temperature were established based on ambient conditions. Leaves were

illuminated by the LED light source mounted on the sensor head. The infra-red gas analyzers (IRGAs) were matched before launching each light response auto-program.

Data analysis

Two-way ANOVA was used to analyze growth measurements, with ramet height, diameter, and leaf number as response variables and treatment and isolate as explanatory variables. Repeated measures MANOVA was used to analyze canker area expansion over time, where levels of the within subject factor (response variable) was canker area over time and the between subjects factors were isolate type and water treatment. G-tests were conducted to test the independence of water treatment from disease incidence and disease-related mortality. Rates of water loss at the beginning and end of the experiment was analyzed with a one-way ANOVA, using sapflow as the response variable and disease incidence as the explanatory variable.

Each light response curve was fit separately with the Mitscherlich function (Potvin *et al.*, 1990) using the NLIN procedure in SAS (SAS Inst. version 9):

$$A=A_{max}\,[1 - e^{-Aqe(PPFD-LCP)}]$$

where A is net photosynthesis, A_{max} is the asymptote of photosynthesis, A_{qe} represents the initial slope of the curve or apparent quantum yield, PPFD is the incident photosynthetic flux density, and LCP is the light compensation point that corresponds to the *x*-intercept (where photosynthetic carbon uptake and respiratory carbon release are in equilibrium). For each light response curve, the adequacy of the Mitscherlich function

was evaluated and consistently showed a good-fit to the data ($r^2 \geq 0.90$). This Mitscherlich function was used to estimate the following parameters: light-saturated rate of photosynthesis (A_{max}), apparent quantum yield (A_{qe}), and the light compensation point (LCP). The slope (A_{qe}) needed to be re-scaled by a factor of 0.0001 due to convergence problems (Peek *et al.*, 2002). Using the Mitscherlich function, the light saturation point (LSP) was calculated as the PPFD where A_{max} was reached. For each light response curve, we also calculated instantaneous water use efficiency (WUE_i= photosynthesis / transpiration) at light saturated values. These LRC parameters, in addition to WUE_i, were analyzed as the response variables in a mixed-model, split-plot ANOVA using the Mixed procedure in SAS. In these analyses, treatment, isolate, and treatment x isolate interactions were included as fixed effects and alder individuals were included as random effects. The Satterthwaite approximation was used for determining the denominator degrees of freedom for hypothesis testing. Although a nonlinear mixed model (NLMixed) approach has been used to analyze photosynthetic response curves (Peek *et al.*, 2002), we were not able to use this approach as NLMixed does not allow for two random statements, which are necessary to estimate the two error terms of a split-plot design.

RESULTS

Water treatment effects

Stomatal conductance measurements indicated that water-limited plants were more water stressed in Trial I (beginning of July) than Trial II (late August). Trial I physiological measurements were taken during conditions of high evaporative demand

(Fig. 3.1a), when air temperature and light ranged between 30-33°C and 622-1116 μmol $m^{-2}s^{-1}$. Water-limited alders in Trial I functioned over a lower range of stomatal conductance (60-80 mmol H_2O $m^{-2}s^{-1}$) than well-watered plants (80-200 mmol H_2O $m^{-2}s^{-1}$) ($F_{1,106}$ = 15.97 P=0.0001). By September, vapor pressure deficit (VPD) had dropped by 50%, temperatures declined by an average of 8 °C, and maximum light intensity was 50% less (483-600 μmol $m^{-2}s^{-1}$) (Fig. 3.1a). The lower driving conditions for evaporative water loss were reflected in decreased rates of transpiration in the well-watered alders of Trial II (1.74 ± 0.13 mmol H_2O $m^{-2}s^{-1}$) vs. Trial I (2.28 ± 0.15 mmol H_2O $m^{-2}s^{-1}$) ($F_{1,108}$=7.46, P=0.0074). In Trial II, stomatal conductance was similar in well-watered (92.0 ± 8 mmol H_2O $m^{-2}s^{-1}$) and the water-limited alders (86.6 ± 7 mmol H_2O $m^{-2}s^{-1}$) (P>0.1).

Monthly measurements of midday and predawn water potential (ψ) also indicated lower water status in water-limited plants. Predawn measurements averaged between -1.43 and- 0.79 MPa in water-limited group vs. -0.49 and -0.39 MPa in the well-watered group ($F_{1,15}$ = 15.76, P=0.0014). Water potential in water-limited plants was typically restricted to lower values (-1.75 to -1.0 MPa) during the day (8:00-17:00 hr), while well-watered alders had higher morning values of ψ (-0.5 MPa) that gradually declined over the course of the day (Table 3.1).

Sapflow

Sapflow decreased over the course of the experiment, in accordance with the decline in vapor pressure deficit. Measurements over the course of nine weeks indicated

higher water loss during Trial I (Fig. 3.1b) compared to August (Fig. 3.1c). We also measured sapflow using two sensors per ramet, with each sensor placed adjacent to either the upper or lower region of a canker. During the midday highs in VPD, between 12:00 and 16:00 hr, gauges on non-diseased alders measured a sapflow difference between 0.09-0.37 g H_2O hr^{-1} compared to a range of 1.11-1.49 g H_2O hr^{-1} for diseased alders ($F_{1,31}$=245.67, P<0.0001)(Fig. 3.2). This indicates that water flowed at a slower rate past the diseased part of the stem. [3]

Plant size

Water-limited plants exhibited reduced plant size in several ways. First, water-limited plants were shorter than well-watered plants by an average of 9 cm in Trial I ($F_{2,82}$=6.19, P=0.015) and by an average of 14 cm in Trial II ($F_{1,59}$=5.45, P=0.232). Second, water-limited plants in Trial II had an average of 31 fewer leaves than well-watered plants ($F_{1,60}$=7.69, P=0.0075), while leaf weight ratios (leaf mass/plant mass)

[3] We dyed the sapwood area around cankers with Safranin O stain and measured the area of functional sapwood every 20 mm extending above and below the canker. The amount of blocked sapwood attributed to the canker extended in a symmetrical fashion from the point of inoculation. On this basis, we assumed that the sapflow gauges, placed at equidistant above and below the canker middle, were associated with a similar amount of functional sapwood area. The flow calculations were based on the diameter of the whole stem and did not account for non-functional sapwood area; therefore, flow was equally overestimated at both locations surrounding the canker.

were similar between treatments (P>0.01). Third, stem diameters of water-limited alders were narrower than well-watered alders by an average of 1.25 mm in Trial 1 ($F_{1,61}$=18.87,P<0.0001) and 2.24 mm in Trial II ($F_{1,57}$=9.06, P=0.0039). These differences resulted in lower mean aboveground biomass (26.63± 1.94 g) in the water-limited group compared to the well-watered group (32.05 ± 1.92 g), ($F_{1,80}$=4.09, P=0.0468). Since plants in both water and isolate treatments had similar leaf specific area (236.00 ± 7.14 cm^2 g^{-1}), we used area-based measurements of photosynthesis for treatment comparisons.

Test of predisposition concept

Trial I. Disease incidence was high in both water treatments and was independent of water treatment (G=0.582, 1 d.f., P=0.445). Eighty-seven percent of Trial I plants developed disease, which was similar to the frequency with which *V. melanodiscus* was re-cultured for both trials (85%). There was only one case of disease-related mortality in Trial I. Canker area steadily increased until 60 to 90 days after inoculation, when the majority of alders developed callusing (70%) (Fig.3.3). Horizontal callus dimensions were greater in well-watered alders (7.86 ± 0.45 mm) compared to water-limited plants (5.74 ± 0.41 mm) ($F_{1,55}$=11.71, P=0.0012). Well-watered plants produced less vertical callus (9.55 ± 0.82 mm) than the water-limited group (13.78 ± 1.02 mm) ($F_{1,55}$=10.21, P=0.0024).

Water-limitation affected disease severity for Isolate 2 alders. During the first trial, disease severity was highest in the water-limited, Isolate 2 alders and peaked 60

days after inoculation (Trial I) (Fig. 3.3). Time-isolate and time-water treatment interactions affected disease severity, but only during in the first trial (Table 3.2). Conidiomata (asexual reproduction) developed during the first five weeks after inoculation, with 13 of the 30 inoculated plants bearing a total of 37 condiomata. Nine of the 13 ramets with condiomata were in the water-limited treatment.

Trial II. In Trial II, water treatment did not affect disease incidence, severity, or disease-related mortality. Ninety-two percent of inoculated plants developed disease symptoms, with 8 inoculations resulting in mortality. Disease-related mortality (G=0.582, 1 d.f., P=0.445) and disease incidence (G=2.09, 1 d.f., P=0.148) were independent of water treatment. The majority of alders (63%) developed callusing, which caused sunken necrotic tissue and decreased canker area from 60 to 90 days after inoculation (Fig.3.3, Table 3.2). Well-watered plants developed more vertical callusing (12.42 ± 6.18 mm) than water-limited plants (9.47 ± 2.42 mm) ($F_{1,55}$=21.22, P <0.0001).

During the second trial, alders inoculated with Isolate 2 generally had higher disease severity than Trial I (Fig.3.3). High conidiomata production reflected the higher disease severity. Conidiomata development peaked approximately five weeks after inoculation when 16 out of 30 ramets bore a total 194 condiomata. A similar number of ramets (6-7) developed conidiomata in each water treatment.

Internal vs. external canker dimensions

The length of external cankers was small (13.1 ± 1.5 mm in Trial I and 12.5± 1.1 mm in Trial II) when compared to the overall length of the stem (935.3 ± 19.2 mm).

However, the length of discolored sapwood was much greater. Each mm of vertical necrosis on the bark surface corresponded to an average of 15.7 mm of pathologically darkened tissue. The length of external cankers was positively correlated with the length of discolored sapwood (r^2=0.32, P=0.0012).

Multiple stressors concept

Light response curve parameters

Trial I. As expected, the highest A_{max} in Trial I was maintained by leaves from control, well-watered ramets (9.13 ± 0.69 μmol CO_2 $m^{-2}s^{-1}$) (Fig. 3.4a, Table 3.3). However, leaves from the water-limited, control ramets (untreated) maintained a similar A_{max} as the water-limited, diseased ramets, indicating that one stress did not exacerbate the other (Fig. 3.5a, Table 3.3). Therefore, similar down-regulatory effects on A_{max} were found in leaves from the ramets that were either water-limited or diseased. These groups all maintained an A_{max} between 6.33 - 6.93 μmol CO_2 $m^{-2}s^{-1}$ (Table 3.4).

We also tested the effects of multiple stressors on other parameters of the light response curve (A_{qe}, LCP, and LSP). In Trial I, the slope of the light response curve (A_{qe}) and the light saturation point (LSP) were affected by disease. For both water treatments, A_{qe} was higher in leaves from diseased ramets. Leaves from the water-stressed, diseased ramets had the highest A_{qe} (109.43 ± 13.76 mol CO_2 mol quanta^{-1}) (Table 3.3, 3.4), in contrast to the lowest A_{qe} measured for the well-watered, control ramets (55.67 ± 12.74 mol CO_2 mol quanta^{-1}). The steep slope and quick curvature of the light response curve led to a low light saturation point for leaves from the water-stressed, diseased treatment

(667 ± 190 μmol $m^{-2}s^{-1}$) (Fig. 3.5a, Table 3.4). However, in leaves from the well-watered control ramets, the lower slope of the light response curve led to a curvature point and light saturation at higher light levels (1643 ±178 μmol $m^{-2}s^{-1}$) (Fig. 3.4, Table 3.4).

Trial II. Trial II did not confirm Trial I results. In contrast, several Trial II alders showed increased photosynthetic performance after inoculation. Trial II light response curves indicated that A_{max} was up-regulated in well-watered ramets treated with Isolate 1 inoculum (10.56 ± 1.29 CO_2 $m^{-2}s^{-1}$) compared to the Isolate 2 ramets (5.75 ± 1.19 CO_2 $m^{-2}s^{-1}$) (Fig. 3.4b, Table 3.4). Isolate 1 was also associated with the smallest cankers in both trials (Fig. 3.3). To confirm the up-regulatory response, we re-measured photosynthetic rates on Trial II plants in October, but did not find the same trend in the up-regulation of well-watered, Isolate 1 plants. We did not detect a water or disease treatment effect for any of the other LRC parameters in the second trial (Table 3.3, 3.4).

Photosynthesis as a function of conductance

Trial I. The water-limited plants photosynthesized over a lower range of conductance values (60-80 mmol H_2O $m^{-2}s^{-1}$). However, leaves from all ramets receiving either the water-limitation or disease treatment in Trial I were restricted to photosynthesis over the lowest values of conductance (Fig. 3.6). Well-watered ramets operated over a higher and broader range of stomatal conductance (67-137 mmol H_2O $m^{-2}s^{-1}$) at light saturation (Fig. 3.6). Diseased ramets from the well-watered treatment maintained a higher instantaneous water use efficiency (WUE_i) (4.90 ± 0.36) than the control ramets (4.09 ± 0.37) (Table 3.3, 3.4).

Trial II. Consistent with the first trial, leaves from the well-watered ramets operated at the highest and widest ranges of conductance values in Trial II. The upper and lower limits of light-saturated stomatal conductance were similar between trials (Fig. 3.6), as well as the range in which the water-limited, diseased ramets operated (60-80 mmol H_2O $m^{-2}s^{-1}$). Also similar between trials was the higher WUE_i in diseased ramets (6.25 ± 0.79) compared to the control ramets (5.07 ± 0.06). Contrary to the results from Trial I, the two pathogenic isolate treatments had opposite effects in the well-watered plants from Trial II. Isolate 2 plants photosynthesized at the lowest end of the range of conductance values of 60 ± 10 mmol H_2O $m^{-2}s^{-1}$, while leaves from Isolate 1 ramets operated at the highest end of the range (150 ± 40 mmol H_2O $m^{-2}s^{-1}$).

DISCUSSION

Predisposition concept

Drought stress has been a working hypothesis for the increasing incidence of Cytospora canker disease on *Alnus* spp. in Alaska (Ruess *et al.*, 2009). At the landscape scale, temperature-induced drought stress and suppressed radial growth in *A. tenuifolia* suggest that summer drought may be associated with increased host susceptibility in *A. tenuifolia* (Ruess *et al.*, 2009; Nossov, 2008). However, drought stress was not related to disease incidence in our study, as the majority of inoculated *A. fruticosa* became infected and developed disease regardless of water treatment. Disease incidence also did not differ between the trials. This was surprising as we expected higher disease incidence during Trial I, when alders were more water stressed and the environment was hotter and drier. Threshold levels of water stress are often required for predisposition to non-aggressive

pathogens (Schoeneweiss, 1975), but our study indicates that the Cytospora pathogen isolates were aggressive enough to infect *A. fruticosa* regardless of water status. Drought stress was also not required for disease predisposition in field studies that inoculated Alaskan hosts, *A. tenuifolia* and *A. fruticosa*, with the same *V. melanodiscus* isolates used in this study (Stanosz *et al.*, 2008; Rohrs-Richey unpublished data).

We also expected disease severity to be higher during the more stressful environment in Trial I; however, severity was highest during the cooler conditions of Trial II. One explanation for higher severity is that environmental conditions may have been more suitable for pathogen growth. Various epidemiological stages typically require specific ranges of temperature and humidity (Berger *et al.*, 1997) and optimal conditions for canker expansion have been measured within the Cytospora genus (Kamiri & Laemmlen, 1981). Optimal conditions for canker expansion are unknown for *Cytospora umbrina* on *Alnus*, but it is possible that the hot, dry conditions of Trial I discouraged canker growth.

Alternatively, there are several lines of evidence indicating that higher disease severity during Trial II was based on the timing of host water stress relative to host phenological stage. First, alders were entering the height of their phenological stage at the beginning of Trial I (July 16th). It is likely that costly defense responses were fully maintained during Trial I, which began just as alders typically enter the peak stage of their phenology (third week of July) when rates of nitrogen fixation and plant growth are the highest (Mitchell & Ruess, 2009). At this stage, higher water status may have

supported additional defensive strategies that can be effective against Cytospora canker, such as increased water supply to the bark and maintenance of cell turgor (Bier, 1953; Bloomberg, 1962).

Trial II alders inoculated with Isolate 2 immediately produced larger cankers, developed more conidiomata, and had higher mortality in response than Trial I alders. Furthermore, the Trial II alders did not produce the healing response of Trial I alders, which had adequate stem growth and callus production to close off the canker almost entirely. This high disease severity during Trial II could be explained by lower active and passive defense responses at later phenological stages. Alders in Trial II were inoculated when alders in the field are typically resorbing nutrients and beginning senescence (Mitchell & Ruess, 2009). During that time, it is likely that resources were not heavily invested in costly processes preventing canker advance, including suberin and lignin production for mechanical barriers (Bloomberg, 1962; Bloomberg & Farris, 1963), non-specific wound healing (necrophylactic periderms and non-suberized impervious tissue) (Maxwell *et al.*, 1997), or synthesis of secondary metabolites (McPartland & Schoeneweiss, 1984; Boyer, 1995).

We can only speculate on the reason for higher disease severity during Trial II. This could be experimentally resolved with an inoculation experiment using a factorial design that crossed levels of phenological stage with different environmental conditions. The environmental parameters of such an experiment would be best informed by more

specific studies on the optimal temperature and humidity ranges required for the epidemiological stages of *Valsa melanodiscus* in Alaska.

Multiple stress concept

We evaluated the multiple stress concept by examining how drought stress and disease influenced host photosynthetic performance. We predicted that well-watered plants challenged by only one stress would maintain a higher light saturated photosynthetic rate (A_{max}) than plants challenged by the simultaneous stresses of water-limitation and disease. As expected, the well-watered, healthy ramets reached the highest A_{max} in Trial I. However, Trial I plants maintained similar values of A_{max} regardless of whether treated with the individual or combined stresses of water-limitation or disease. These Trial I results indicate that one type of stress did not exacerbate the other; rather, the stresses resulted in a generalized depression in A_{max}. These results do not support the multiple stress concept but instead suggest that reduced A_{max} reflected systemic down-regulation and generalized stress response from both water stress and the localized stem canker (Chapin, 1991; Isaac, 1992; Flexas *et al.*, 2004).

Although A_{max} did not reflect a multiple stress response, the light response parameters A_{qe} and LSP did support the multiple stress concept for Trial I. Leaves of well-watered, healthy ramets reached light saturated photosynthesis at light intensities that were more than double the light intensity at which the leaves from water-limited, diseased ramets reached light saturation. The low LSP measured for the water-limited, diseased ramets was achieved by high A_{qe} (steep slope) and quick curvature of the light

response curve. Low LSP for the water-limited, diseased ramets likely reflects stomatal limitations as well as metabolic limitations to carbon fixation, as these leaves operated over a range of conductance values below the threshold level (100 mmol H_2O $m^{-2}s^{-1}$) at which RuBP regeneration is considered to be resistant to water stress (Flexas *et al.*, 2004). Low LSP is also indicative of the inability to use high light intensities, which can increase the risk of photoinhibition in water-limited, diseased ramets during daily maxima of light and temperature (Ayres, 1984). Low intensity saturation has been found previously for diseased plants (Niederleitner & Knoppik, 1997) and suggests that water stress and disease can mechanistically limit the ability to fix carbon in addition to risking photosystem damage under high light, high temperature conditions.

We only detected down-regulation of light response in water stressed, diseased plants during Trial I. Treatment effects may have been easier to detect during Trial I, since it overlapped with peak phenology when plants operate close to physiological potential. During this stage, we captured the reduction in LSP and A_{max} under drought stress and disease, a mechanistic explanation of how carbon resources are limited for water stressed alders with Cytospora canker.

Despite later phenology during Trial II, light parameters during this trial suggest an important mechanism by which alders may compensate for disease. We measured up-regulation of A_{max} in well-watered ramets inoculated with Isolate 1, which maintained an A_{max} twice that of those treated with Isolate 2. Alders may have upregulated A_{max} for two reasons. First, the low disease damage associated with Isolate 1 could have allowed

compensatory photosynthesis in the host. Alternatively, the Isolate 1 pathogen may have placed a higher metabolic demand on its host and plants responded by up-regulating photosynthesis. Photosynthetic upregulation can be a compensatory response to the earlier stages of fungal infection and colonization, when the host may be able to support the increased carbon costs associated with pathogen biomass (Lucas, 1998; Isaac, 1992). Upregulation of A_{max} may also be a mechanism by which plants tolerate this disease. Since up-regulation was only found in well-watered plants, this suggests that water availability may affect the capacity for compensatory photosynthesis and potential tolerance in response to disease. However, even in the well-watered alders, upregulation was a temporary response (it was not found in measurements 2 weeks later) which was not sustained during later phenological stages.

Stomatal regulation of water loss

In addition to the effects of water stress and disease on light response parameters, the canker disease also decreased the amount of functional sapwood tissue and reduced water transport during daily periods of high vapor pressure deficit. Pathogen colonization of the vascular system can decrease functional sapwood by causing resistance to water flow, interfering with osmotic gradients, or blocking and embolizing conduits (Ayres, 1981; Sutic & Sinclair, 1991), all of which may be exacerbated by water stress. We found that alders consistently used stomatal regulation to ameliorate the interference of cankers with water transport, as diseased ramets in both trials consistently had higher peak instantaneous water use efficiency (WUE_i) than healthy ramets. In Trial I and II, we

found that leaves from the water-limited, diseased ramets operated within a narrow range of stomatal conductance values (63 -76 mmol $m^{-2}s^{-1}$). This range is much lower than maximum conductance values in our experiment (137-146 mmol $m^{-2}s^{-1}$), the range of stomatal conductance values previously reported for water stressed alders (181-268 mmol $m^{-2}s^{-1}$) (Hibbs *et al.*, 1995; Schrader *et al.*, 2005), and the typical range for woody plants (Eschenbach & Kappen, 1999). Stomatal regulation is not necessarily a given in alders (e.g., *A. glutinosa*, Eschenbach & Kappen, 1999) or in diseased plants (Ayres, 1981). Our study indicates that stomatal regulation is generally used as a disease-coping strategy for *A. fruticosa*, whereas photosynthetic upregulation appears to be a strategy conditional on water status. Since plant pathogens influence all physiological processes throughout the plant (Isaac, 1992; Sutic & Sinclair, 1991; Lucas, 1998), the capacity for these types of adjustments in physiological performance may buffer individuals against the effects of multiple stresses (Helmuth *et al.*, 2005).

Conclusions

Our results are not entirely aligned with the general assumption that climate-related stressors will physiologically compromise plants and reduce their capacity to defend against or recover from disease damage (Larsson, 1989; Mitchell *et al.*, 2003; Rodriguez *et al.*, 2004). In our study, the highest disease damage did not correspond to the most stressful environmental conditions. Instead, disease severity was highest in alders inoculated during later phenological stages (Trial II) and under a less stressful environment. The most suppressed disease levels were in Trial I, well-watered alders,

which were inoculated during peak phenological stage. These alders experienced the most demanding environmental conditions and had lower physiological performance under the simultaneous stresses of water-limitation and disease. Directional changes in temperature may be the primary driver behind changes to plant-pathogen dynamics; however, the dependence of our results on host phenological stage and environment makes it difficult accept that increased temperatures will lead to higher levels of disease for this pathosystem.

ACKNOWLEDGEMENTS

This research was supported by grants to J.K. Rohrs-Richey from the Arctic Institute of North America, the Center for Global Change and Arctic System Research, and fellowships from Alaska's Experimental Program to Stimulate Competitive Research (EPSCoR). This research was partially funded through a grant to Barbara A. Roy and Christa P.H. Mulder, supported by the Office of Science, Biological, and Environmental Research Program (BER), U.S. D.O.E., through the Western Regional Center of the National Institute for Global Environmental Change (NIGEC), under Cooperative Agreement No. DE-FC02-03ER63613. The research greenhouse was managed by Heather McIntyre. Research assistance provided by Michele Burrell. *Valsa melanodiscus* isolates were obtained by Gerald Adams, Michigan State University.

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Table 3.1 Leaf-level measurements indicating water treatment effects. For the water-limited ($-H_2O$) and well-watered ($+ H_2O$) groups, an example of the daily fluctuation in water potential (ψ; MPa) is shown from 8:00 to 19:00 hr on July 18th when water potential measures were taken after a short period (2 days) of no water in the water-limited ($-H_2O$) treatment (n=3 for each time period). The standard protocol for the $-H_2O$ treatment was the application of low volumes of water for an average of 4 days followed by a short period (1-2 days) of no water. After the measurement at 18:00 hr, plants were watered and water potential was fully restored to early morning values in the $+ H_2O$ group. Stomatal conductance (g_s; mmol m^{-2} s^{-1}) and transpiration (E; mmol m^{-2} s^{-1}) measurements (on healthy controls) are also shown for July and August after a similar period of withheld water followed by restored water (n=8-10). Means ± SE.

Trtmnt	ψ					g_s		E	
	8:00	12:00	16:00	18:00	19:00	July	Aug.	July	Aug.
$+H_2O$	-0.47	-0.72	-0.90	-0.92	-0.47	199	81.2	3.99	1.79
	(0.06)	(0.07)	(0.05)	(0.08)	(0.06)	(24)	(11)	(0.36)	(0.22)
$-H_2O$	-1.27	-1.43	-1.53	-1.40	-0.65	118	67.3	2.88	1.89
	(0.23)	(0.12)	(0.20)	(0.10)	(0.06)	(28)	(10)	(0.56)	(0.18)

Table 3.2 Repeated measures MANOVA on canker area. Time contrasts shown for the effects of water treatment (Trtmnt), isolate type (1,2), and time on canker area. MANOVA tests use Roy's Greatest Root with 4 numerator degrees of freedom and 47 denominator degrees of freedom. Significance level; *** P< 0.001; **P<0.01; *P<0.05; NS= not significant.The Greenhouse-Geisser Epsilon Adjustment was used to adjust degrees of freedom for within subject tests.

	Trial I		Trial II	
Source	F-value		F-value	
Time	59.12	***	8.22	***
Time*isolate	3.11	*	1.60	NS
Time*trtmnt	3.08	*	1.66	NS
Between Subject				
Isolate	0.24	NS	6.69	**
Trtmnt	6.02	*	2.56	NS
Within Subject				
Time	125.79	***	11.33	***
Time*isolate	4.22	**	2.84	NS
Time*trtmnt	6.67	***	0.95	NS

Table 3.3 The effects of treatment and isolate on light response curve parameters. Results from the mixed-model, split-plot ANOVA on the effects of treatment (Trtmnt) and isolate type (1, 2). A_{max}, the light saturation point (μmol CO_2 $m^{-2}s^{-1}$); A_{qe}, the quantum efficiency (mol CO_2 mol^{-1} quanta); LCP, the light compensation point (μmol $m^{-2}s^{-1}$); LSP, light saturation point (μmol $m^{-2}s^{-1}$); and WUE_i, water use efficiency((μmol CO_2 $m^{-2}s^{-1}$(μmol H_20 $m^{-2}s^{-1}$)). In Trial I, statistical differences could not be detected between isolates, so they were pooled. Significance level; *** P< 0.001; **P<0.01; *P<0.05; NS= not significant. Degrees of freedom were approximated using the Satterthwaite method.

		Trial I				Trial II			
Variable	Effects	Num. d.f.	Den. d.f.	F-value		Num. d.f.	Den. d.f.	F-value	
A_{max}	Trtmnt	1	11	2.77	NS	1	8.52	0.00	NS
	Isolate(s)	1	11	6.41	*	2	11.3	2.36	NS
	Trtmnt*Isolate	1	11	2.36	NS	2	11.3	5.43	*
A_{qe}	Trtmnt	1	11	3.62	NS	1	9.37	0.02	NS
	Isolate(s)	1	11	5.21	*	2	12	1.29	NS
	Trtmnt*Isolate	1	11	0.15	NS	2	12	1.49	NS
LCP	Trtmnt	1	11	0.89	NS	1	7.2	2.42	NS
	Isolate(s)	1	11	2.53	NS	2	10.8	1.81	NS
	Trtmnt*Isolate	1	11	0.23	NS	2	10.8	0.84	NS
LSP	Trtmnt	1	11	8.07	*	1	8.72	0.46	NS
	Isolate(s)	1	11	1.73	NS	2	10.7	0.70	NS
	Trtmnt*Isolate	1	11	0.33	NS	2	10.7	0.70	NS
WUE_i	Trtmnt	1	11.1	0.99	NS	1	8.9	0.04	NS
	Isolate(s)	1	11.6	9.77	**	2	10.9	6.81	*
	Trtmnt*Isolate	1	11.6	6.09	*	2	10.9	0.03	NS

Table 3.4 Estimates of light response curve parameters and WUE_i. Values for Amax ($\mu mol\ CO_2\ m^{-2}s^{-1}$), A_{qe} ($mol\ CO_2\ mol^{-1}$ quanta), LCP ($\mu mol\ m^{-2}s^{-1}$), LSP ($\mu mol\ m^{-2}s^{-1}$), and WUE (($\mu mol\ CO_2\ m^{-2}s^{-1}$($\mu mol\ H_20\ m^{-2}s^{-1}$)) are least square means estimates with standard errors in parentheses. For Trial I, both isolate types (1,2) were pooled (Isolate 1) for statistical tests. Otherwise, control=0, Isolate 1=1, Isolate 2=2. Tests for differences between means based on the Tukey-Kramer adjustment in the ANOVA mixed procedure. Significant differences at the α=0.05 level for water- isolate combinations are indicated by letters.

Trial	Trtmnt	Isolate	A_{max}	A_{qe}	LCP	LSP	WUE_i
1	$-H_2O$	0	6.87 (0.74)	89.11 (13.76)	18.74 (4.53)	833 (220)	4.97 (0.39)
	$-H_2O$	1	6.33 (0.74)	109.43 (13.76)a	10.31 (4.53)	667 (190)a	5.06(0.39)
	$+H_2O$	0	9.13 (0.69)b	55.67 (12.74)b	12.41 (4.19)	1643 (178)b	4.09 (0.37)a
	$+H_2O$	1	6.93 (0.69)a	84.30(12.74)	7.87 (4.19)	1214 (301)	4.90 (0.36)b
2	$-H_2O$	0	9.49 (1.06)	65.02 (13.11)	9.93 (2.56)	1000 (209)	5.07 (0.55)a
	$-H_2O$	1	7.09 (1.32)	83.08 (16.28)	7.98 (3.27)	1200(200)	5.72 (0.67)b
	$-H_2O$	2	7.02 (1.58)	89.15 (19.53)	9.31 (3.98)	---	6.25 (0.79)b
	$+H_2O$	0	7.41 (0.93)	79.87 (11.57)	14.94 (2.22)	1250 (122)	5.28 (0.49)a
	$+H_2O$	1	10.56 (1.29)a	57.05 (16.06)	7.79 (3.26)	1250 (120)	5.84 (0.65)b
	$+H_2O$	2	5.75 (1.19)b	94.09 (14.82)	17.53 (2.94)	---	6.17 (0.61)b

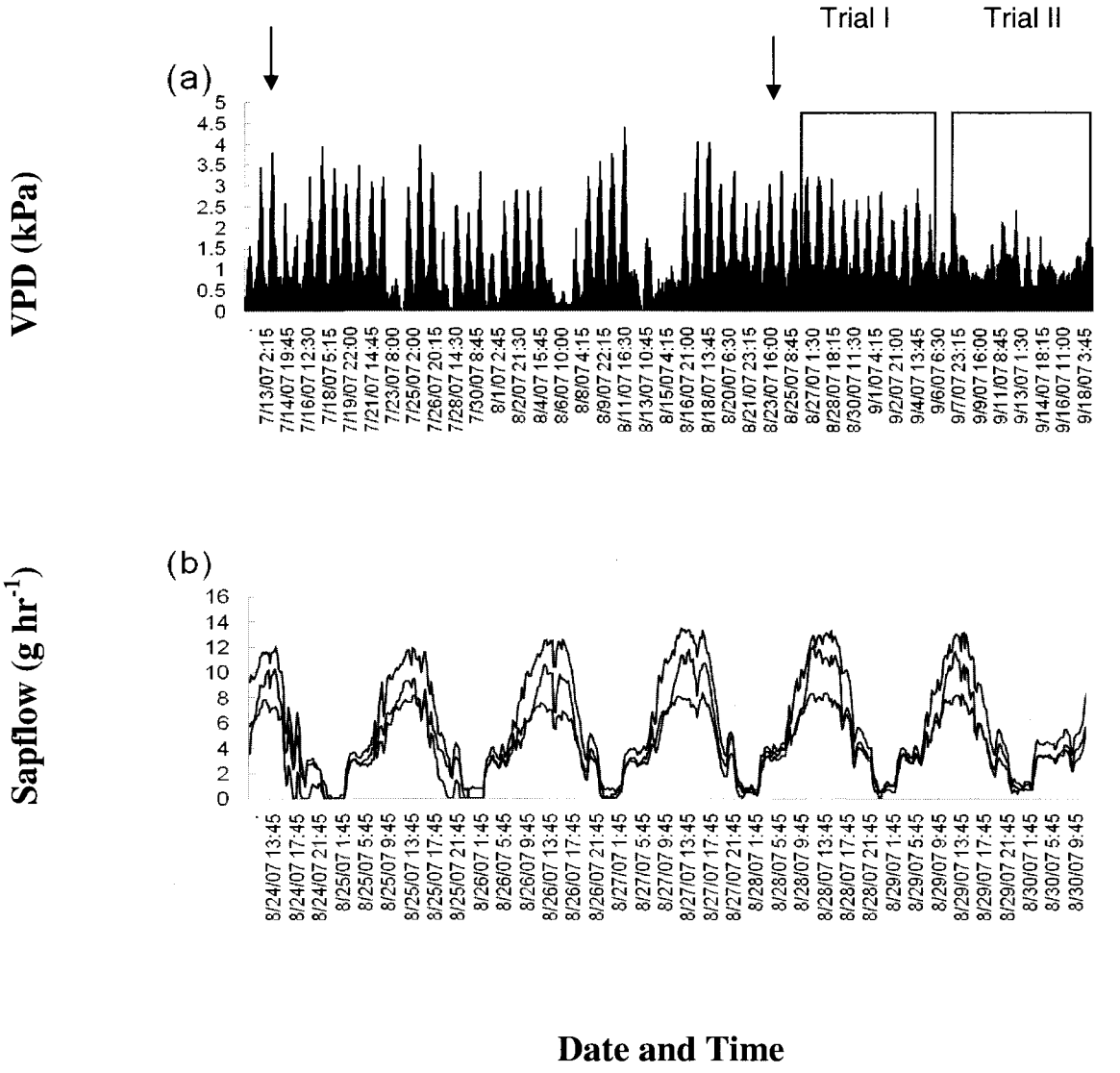
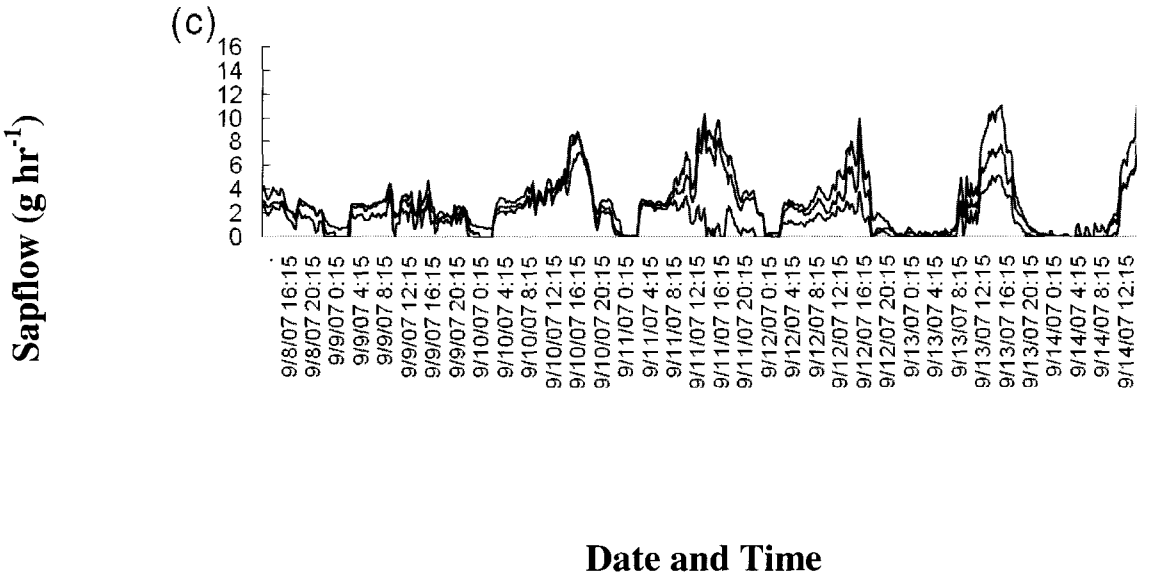


Fig. 3.1 Greenhouse vapor pressure deficit and sapflow. Arrows on VPD graph (a) indicate the dates on which inoculation began in Trial I and Trial II. The squares on (a) enclose the time period during which light response curves were measured for Trial I and II. For these time periods, corresponding sapflow in healthy alders combined is shown in the lower graphs (b,c), depicting differences in plant water loss (n=3 for each period of sapflow).

Fig. 3.1 continued.


(c)
Sapflow (g hr-1)
16
14
12
10
8
6
4
2
0
9/8/07 16:15
9/8/07 20:15
9/9/07 0:15
9/9/07 4:15
9/9/07 8:15
9/9/07 12:15
9/9/07 16:15
9/9/07 20:15
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Date and Time

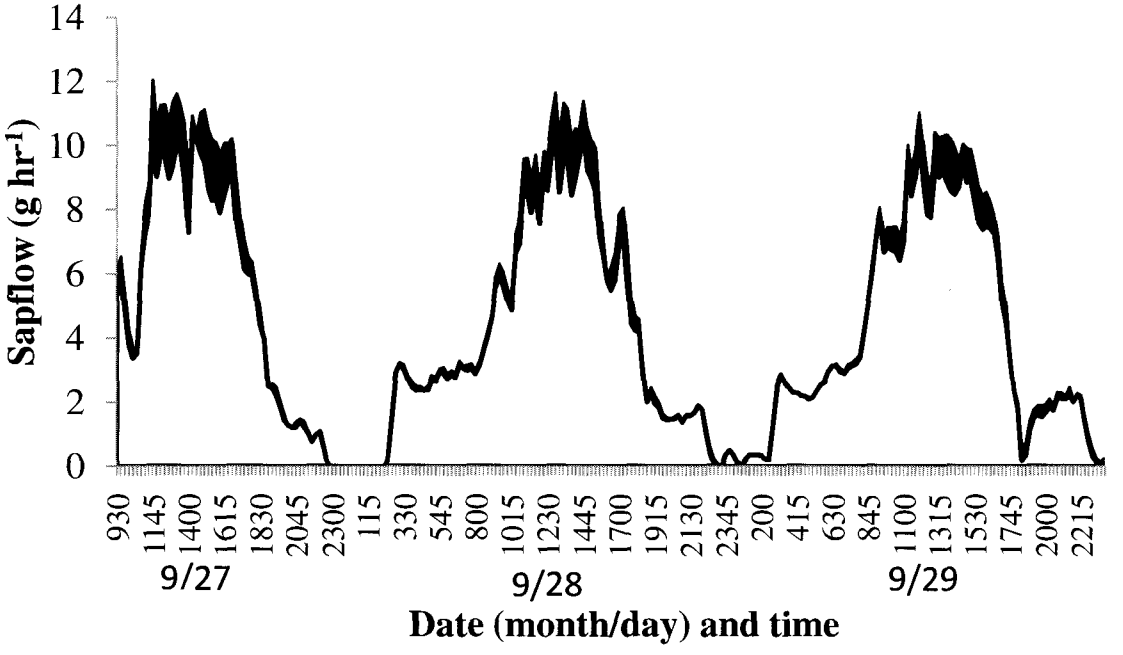


Fig. 3.2 Paired sapflow measurements from adjacent regions to a stem canker. The curves represent sapflow over three days. The area of each curve is divided into two parts. The white area represents the velocity of water transported above the canker. The dark portion of the curve represents the velocity water transported just below the canker, indicating the difference in sapflow velocity between the upper and lower regions adjacent to the canker. The greatest differences in sapflow velocity between the gauges occurs during peak sapflow, when driving variables (light, temperature, vapor pressure deficit) are high.

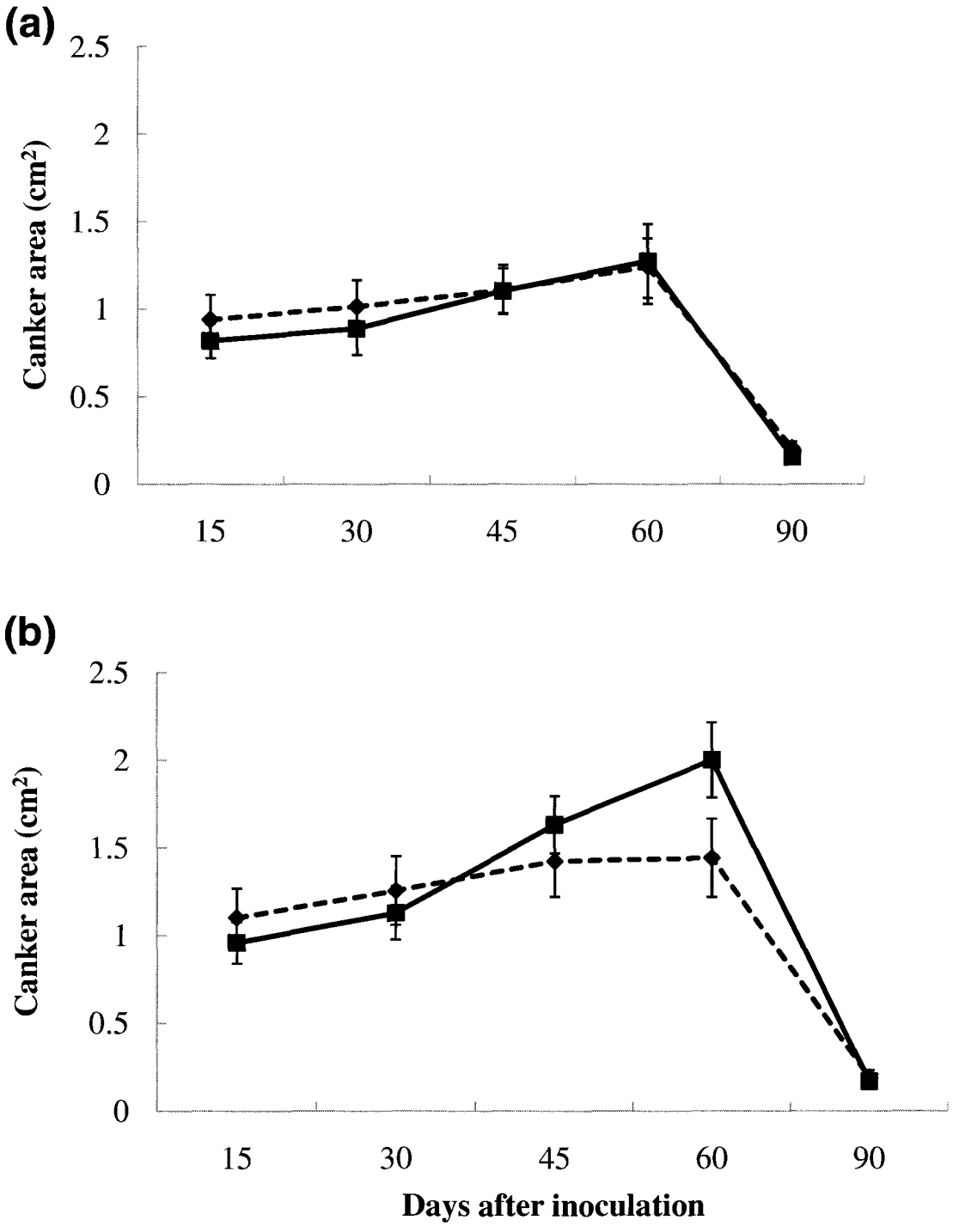


Fig. 3.3 Changes in canker area. Area shown for Trial I (a) well-watered and (b) water-limited plants. In Trial II, area is shown for (c) well-watered and (d) water-limited plants. Each time period after inoculation shows mean canker area ± 1 S.E. for Isolate 1 (dotted line) and Isolate 2 (solid line) (n=15 for each isolate).

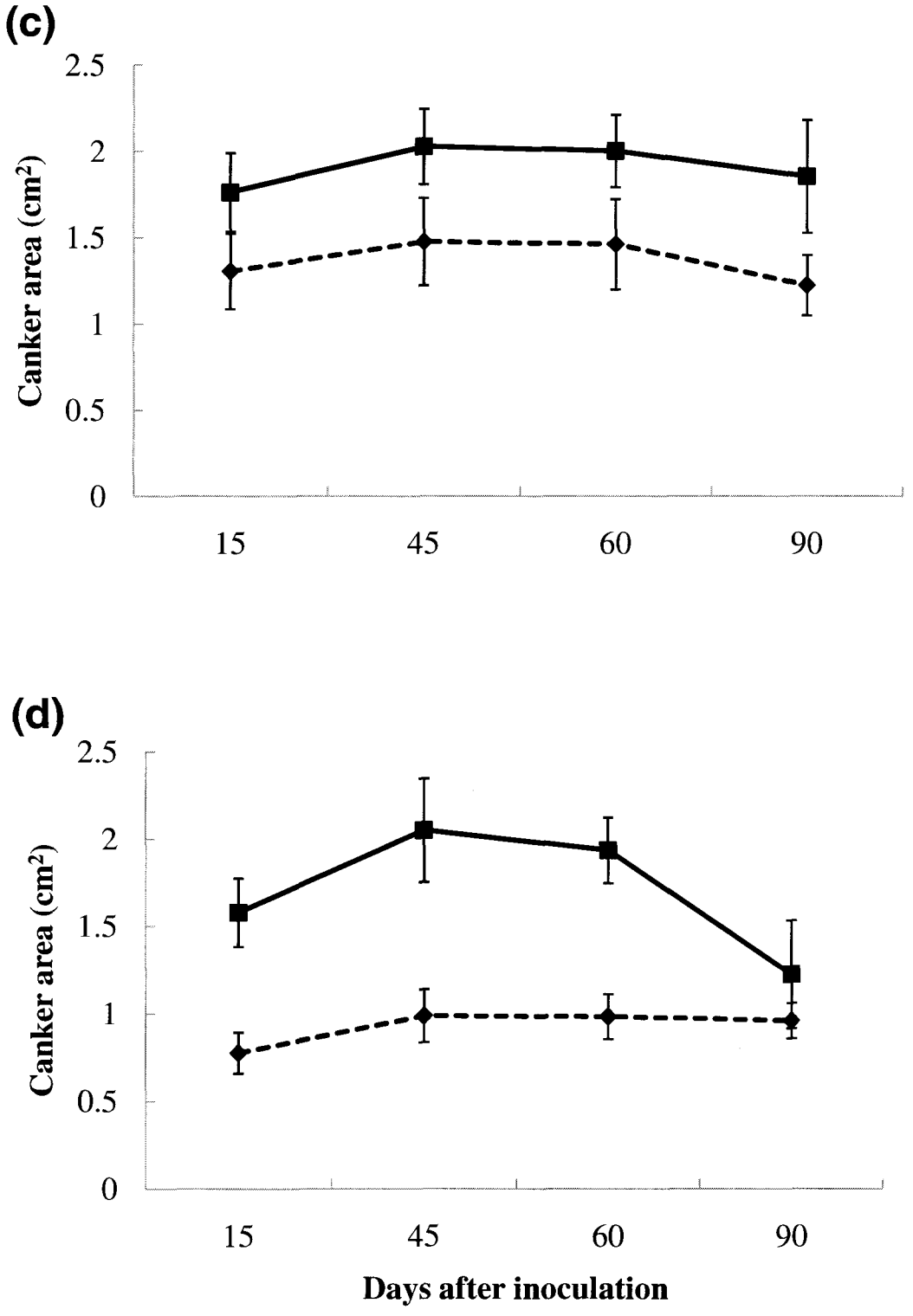


Fig. 3.3 continued.

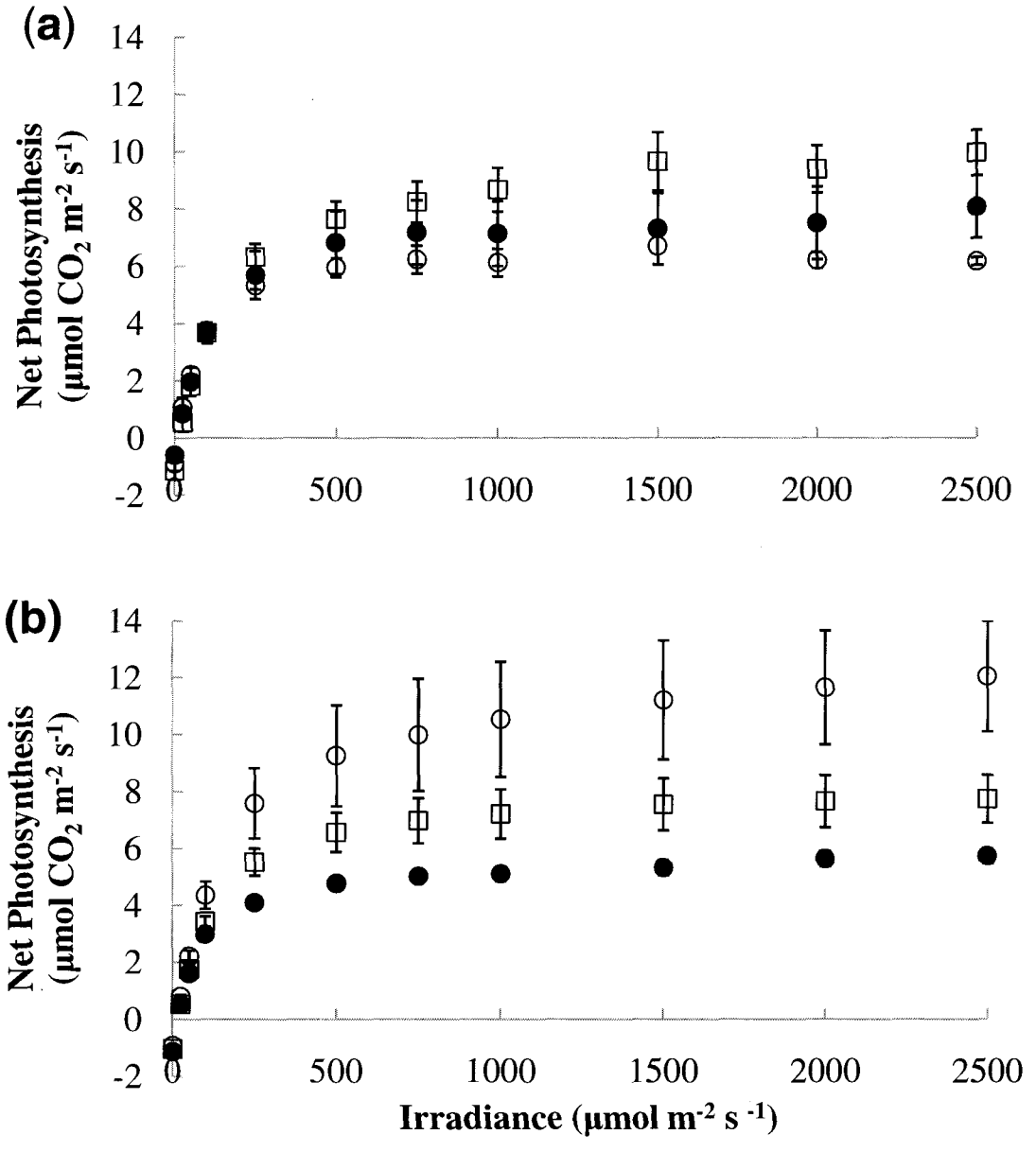
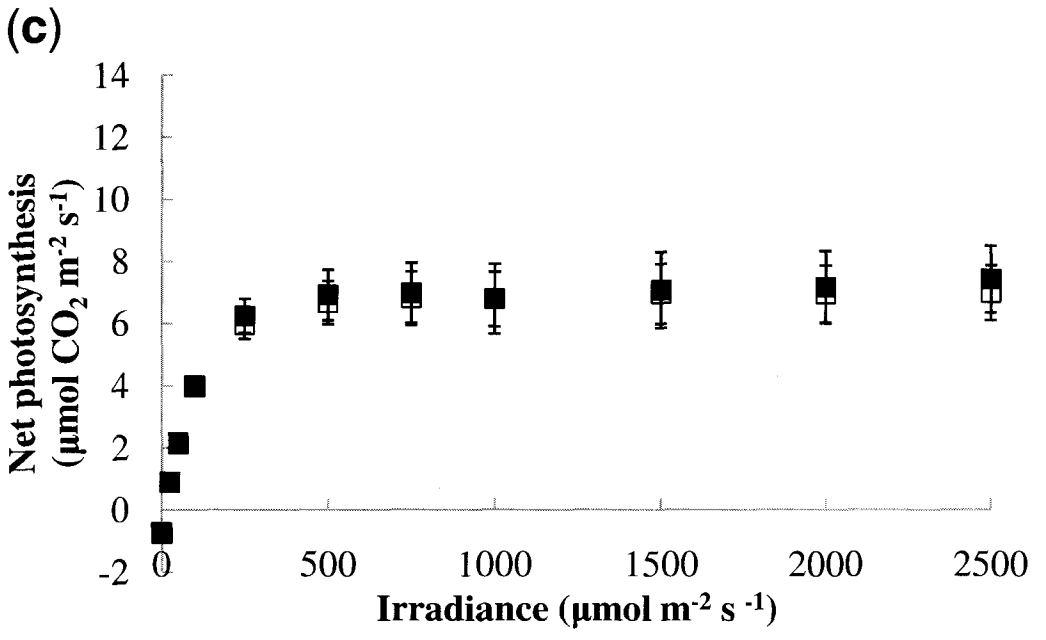


Fig. 3.4 Light response curves for the well-watered treatment. Shown for the Trial I (a), Trial II (b), and the no-disease control group (c). Measurements are based on a split-plot design, where water treatment is applied at the whole-plot level (alder) and isolate type is applied at the sub-plot level (ramet). Each point is the mean ± 1 S.E from ramets treated with either Isolate 1 (○), Isolate 2 (●), or untreated, control ramets (□). In the no-disease control groups for August and September, light response curves were not different between the untreated ramet (□) and the ramet treated with wound + agar only (■). Therefore, only the September group (c) is shown (n=3 ramets for each isolate and n=6 ramets for controls).



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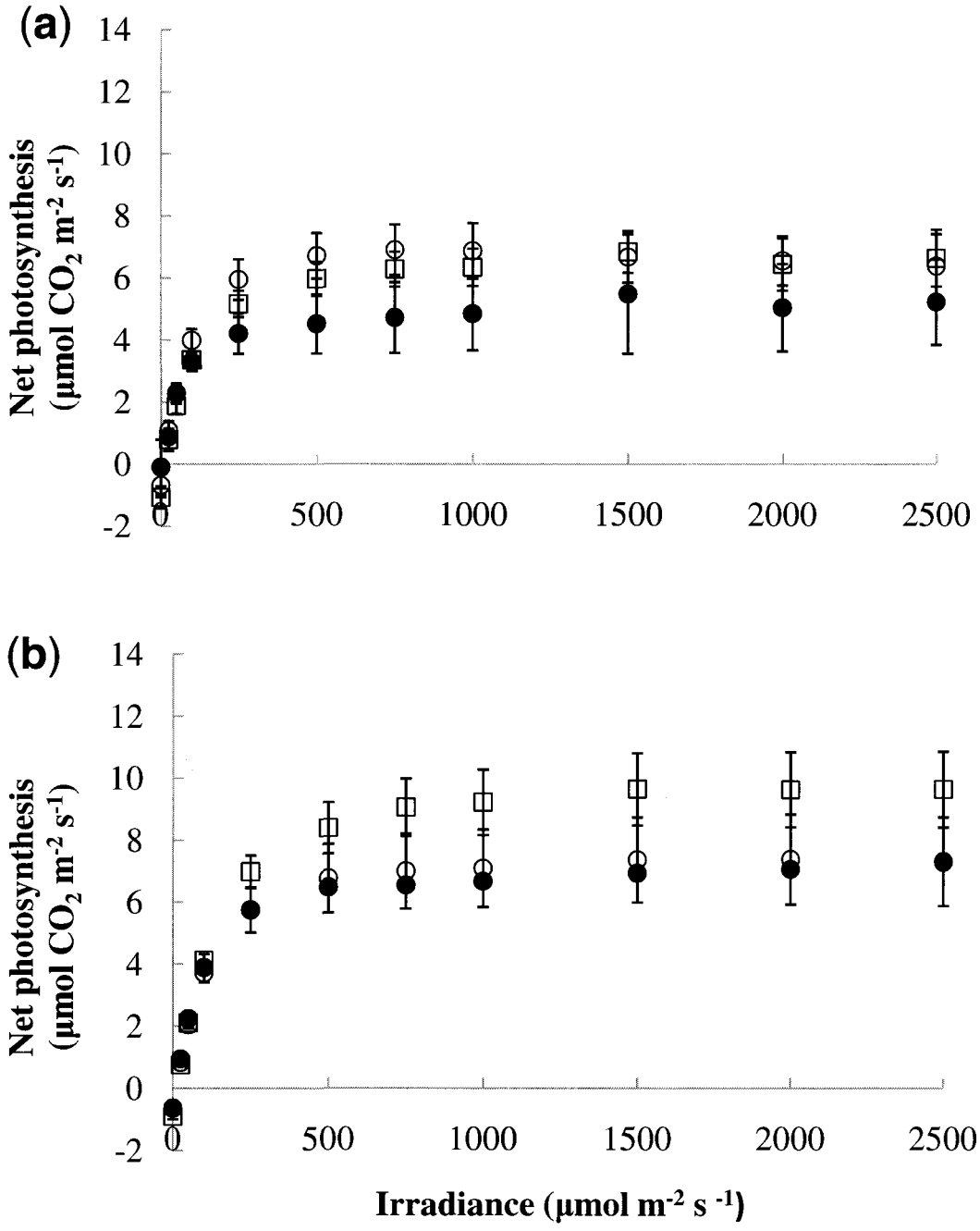

Fig. 3.5 Light response curves for the water-limited treatment. Results shown for Trial I (a), Trial II (b), and the September no-disease control group (c). Each point is the mean ± 1 S.E from ramets treated with either Isolate 1 (○), Isolate 2 (●), or untreated, control ramets (□). In the no-disease control groups for September, light response is shown for the untreated ramet (□) and the ramet treated with wound + agar only (■).

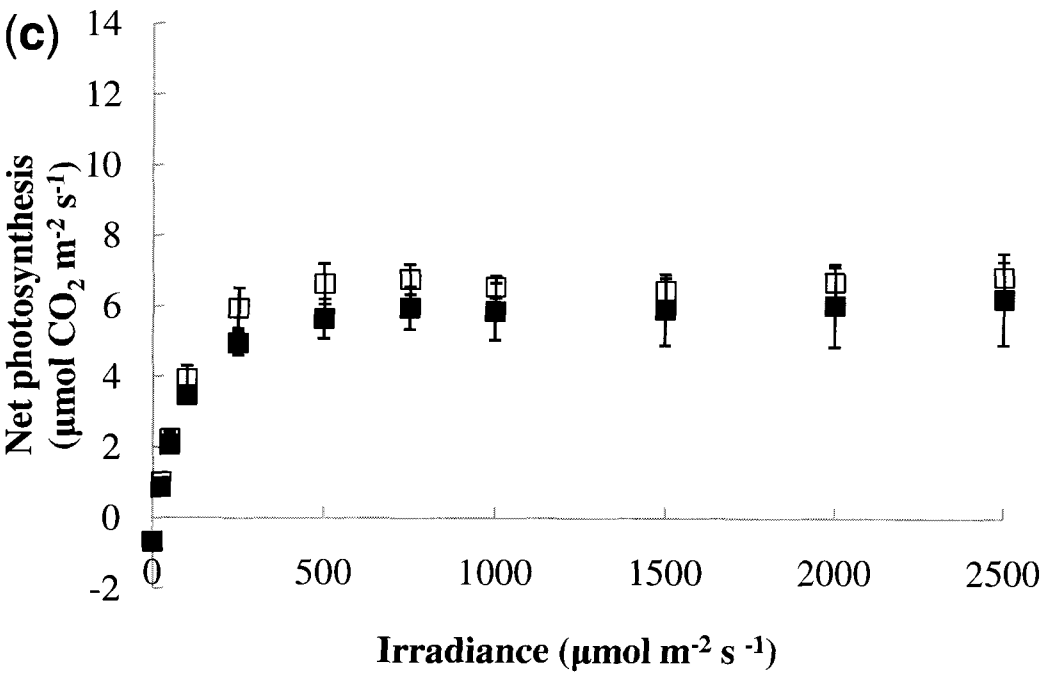

Fig. 3.5 continued.

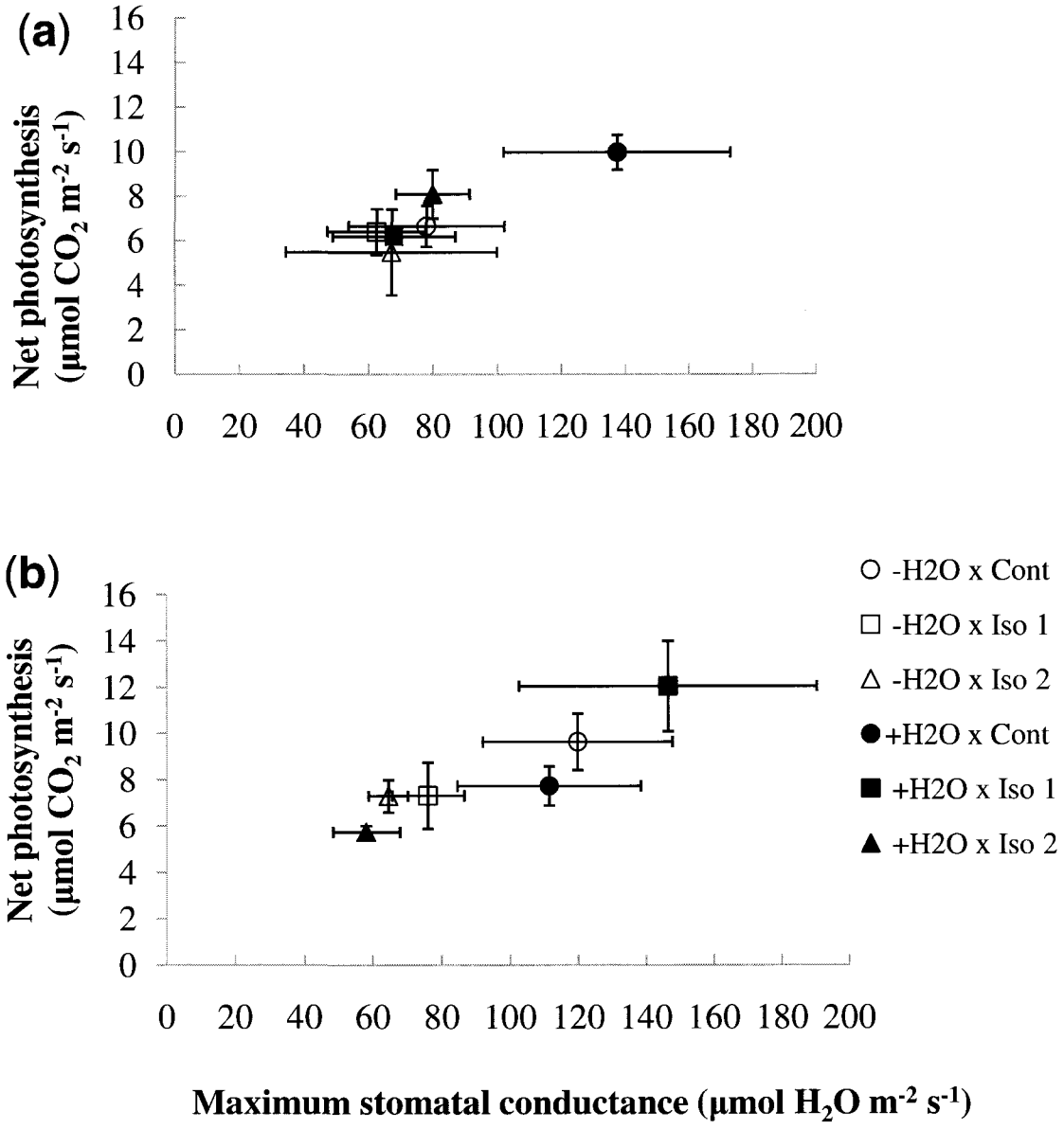

Figure 3.6 Net photosynthesis as a function of maximum stomatal conductance. Shown for Trial I (a) and II (b). At maximum stomatal conductance under the highest irradiance level, the mean photosynthetic rate ± 1 S.E. is plotted as a function of mean stomatal conductance ± 1 S.E for each water treatment and isolate combination (n=3 for each isolate and n=6 for controls).

CHAPTER 4

ENHANCED WATER LOSS FOLLOWING HERBIVORY-RELATED DECLINE IN LEAF AREA: SAPWOOD AREA RATIO IN THE ALASKAN SHRUB *ALNUS FRUTICOSA*[4]

ABSTRACT

The impact of herbivory on plants in natural systems is typically quantified as the amount of net primary production removed on an annual basis. However, this measure can be misleading because the impact of herbivory on plants may not be directly related to the amount of foliage removed. One reason is that plants can often compensate for herbivory, thus, the amount of tissue loss does not directly translate to similar changes in plant function. Our study addresses this discrepancy by using a plant architectural index (leaf area: sapwood area) to examine changes in plant function following herbivory. For the boreal shrub *Alnus fruticosa* we investigated whether changes to the ratio of leaf area to sapwood area (LA/SA) following herbivory were related to the capacity for transpirational water loss. For *A. fruticosa* with low mean LA/SA (0.11 ± 0.01 m^2/cm^2) the mean rate of leaf area-based daily water loss was approximately twice that of alders with medium (0.27 ± 0.01 m^2/cm^2) or high LA/SA (0.46 ± 0.03 m^2/cm^2). Levels of herbivore damage were directly related to enhanced rates of water loss, but the post-

[4] Rohrs-Richey, J.K., Mulder, C.P.H., Roy, B.A. Enhanced water loss following herbivory-related decline in leaf area: sapwood area ratio in the Alaskan shrub *Alnus fruticosa*. Prepared for: Journal of Functional Ecology.

herbivory LA/SA ratio explained more variation in leaf area-based water loss. Therefore, while the impacts of herbivory can be related to the amount of tissue loss, our study suggests that because of physiological compensation, the post-herbivory LA/SA ratio is a more functionally relevant measure of the impact of herbivory on woody species such as *A. fruticosa*.

INTRODUCTION

The impact of herbivory on plants in natural systems is often quantified by the amount of net primary production removed per year, which can range from 2 to 15% in forests (Zangerl et al. 2002). However, the amount of tissue taken by herbivores does not directly translate to a similar magnitude of change in plant function (Welter 1989), and this makes it difficult to predict the impact of herbivores on individual plants and plant communities (Zangerl et al. 2002). One reason for this unpredictability is that leaf damage can be off-set or buffered by integrated responses within the whole plant that can compensate for loss of functional leaf area (Baldwin and Preston 1999, Bucci et al. 2004). For example, studies measuring whole-plant transpiration following partial defoliation commonly show enhanced rates of water loss per unit area of the remaining foliage (Meinzer and Grantz 1990, Pataki et al. 1998, Tausend et al. 2000). Increased rates of transpiration are related to increased stomatal conductance, which presumably increases photosynthetic performance and is a common response to defoliation (Meinzer and Grantz 1990, Pataki et al. 1998, Tausend et al. 2000). Since the enhancement of leaf area-based physiology in the remaining foliage can minimize the total impact of pest damage on plant function (Jones and Coleman 1991), the amount of leaf area damage may not be a realistic measurement of herbivore impact on plant function.

We use an index of plant architecture (leaf area: sapwood area) to evaluate changes to plant water relations following herbivory. The ratio of leaf area per unit of supporting sapwood area (LA/SA) approximates the relationship between the evaporative

demand of the canopy and the hydraulic capacity of the bole (Preston and Ackerly 2003) and is thus an architectural index of supply and demand (Tausend et al. 2000, Bucci et al. 2004, Bucci et al. 2005). Changes to this ratio can have direct implications for plant performance following herbivory. For example, a plant with lower LA/SA has reduced canopy water demand relative to the supplying sapwood area (Sala et al. 2001, Bucci et al. 2004, Choat et al. 2007). The lower LA/SA can support enhanced rates of water loss per unit leaf area and a higher capacity for carbon uptake and assimilation in the remaining foliage. This is presumably achieved by tightly coordinated increases in stomatal conductance and hydraulic conductance (Meinzer and Grantz 1990, Pataki et al. 1998, Tausend et al. 2000). Therefore, we use this index of supply and demand to evaluate the functional changes accompanying herbivory. Considering the impact of herbivores on the integrated parts, rather than just the leaf area, can reflect the capacity for physiological responses to herbivory.

Our study focuses on the effects of herbivory on *Alnus fruticosa*, a boreal shrub that does not typically replace foliage following herbivory (Bryant and Kuropat 1980); therefore, herbivory often results in permanent changes to plant architecture for the remainder of the growing season. *A. fruticosa* has a slower rate of growth, photosynthesis, and nutrient absorption than several co-occuring boreal species (*Betula papyrifera*, *Populus tremuloides*, *Salix* sp.) (Chapin et al. 1986). These strategies may allow *A. fruticosa* to be persistent into late succession and dominant at latitudinal and altitudinal treeline (Mitchell and Ruess 2009). However, these same strategies also constrain its capacity to replace foliage following herbivory, even in high light and fertile

soils (Bryant and Kuropat 1980, Bryant et al. 1983, Bryant et al. 1987, Chapin 1991). Furthermore, *A. fruticosa* supports additional carbon and nutrient costs associated with its symbionts (the N-fixing *Frankia* and ectomycorrhizae), which impose further constraints on re-growth following partial defoliation (Ruess et al. 2006).

Our study uses a partial defoliation event on *A. fruticosa* in the Alaskan interior to assess the consequences of herbivory-related changes in LA/SA for plant function. In June 2005, the levels of foliar insect chewing damage exceeded the cumulative amount of herbivore damage (~15%) that alders typically experience throughout an entire growing season (Mulder et al. 2008) (Fig. 4.1). During this event, the alders included in our study experienced a variable amount of canopy loss from leaf chewing, with losses ranging from 5% to 83 % canopy area. Consequently, the partial defoliation resulted in a 10-fold difference in the ratio of leaf area to sapwood area (LA/SA). Following this early season defoliation, we measured transpirational water loss and leaf water potential throughout the summer to gauge how herbivore damage translated to functional changes in *A. fruticosa*.

First, we assessed the physiological performance of *A. fruticosa* according to herbivory related shifts in the LA/SA ratio. We expected post-herbivory LA/SA ratio to explain more variation in transpirational water loss than the amount of tissue removal from herbivore damage. Since a lower LA/SA ratio implies a higher capacity for water transport (sapwood area) relative to the water demand (leaf area) (Sala et al. 2001, Choat

et al. 2007), our first hypothesis was that alders with lower LA/SA would support higher leaf area-based transpiration rates.

Second, we evaluated the consequences of herbivory-related changes in LA/SA for transpirational water loss during a seasonal decline in site-level soil moisture. With a decrease in LA/SA ratio, the lower water demand (canopy area) relative to water supply (sapwood area) can prevent the development of damaging water potential gradients (McDowell et al. 2002). Therefore, our second hypothesis was that low LA/SA plants would maintain higher transpirational water loss during soil drying, while alders with higher LA/SA ratios may be forced to increase stomatal regulation and restrict rates of water loss. We evaluated LA/SA related changes in water loss using parameters of diurnal sapflow curves, which can indicate the capacity for water transport under high vapor pressure deficit (VPD) and the responsiveness to VPD.

Third, we evaluated leaf water potential and liquid phase hydraulic conductance in relation to LA/SA ratios. Our third hypothesis was that if alders increased transpiration rate on a leaf area basis while also maintaining leaf water potential, there would be an accompanied increase in liquid phase hydraulic conductance (the ratio of transpiration per unit leaf area to the gradient in water potential) at lower LA/SA ratios (Meinzer and Grantz 1990, Bucci et al. 2004).

METHODS

Site selection

This study took place in the boreal forest of interior Alaska, which has a semi-arid, continental climate with 280 mm precipitation annually and a mean annual temperature of -2.94 °C (www.climate.gi.alaska.edu/Climate). We conducted our study at sites that were part of a longer-term study (2001-2008) that evaluated cumulative herbivore and pathogen damage on *A. fruticosa* across a local climate gradient (Mulder et al. 2008, Mulder and Roy unpublished data). From this climate gradient, we selected a subset of seven sites based on the range of monthly mean summer air temperatures that were measured from 2003-04 (Table 4.1). All sites had a narrow range of winter air temperatures (-27.35 to -29.31 °C from December to February). Research plots at sites were 50 m in diameter and located in mixed stands of predominantly white spruce (*Picea glauca* (Moench) Voss), birch (*Betula neoalaskana*) or aspen (*Populus tremuloides* Michx.). These upland, secondary successional stands had similar stand characteristics: dense canopy cover (76-79%), low coverage by mosses and lichens (0-2 % ground cover), and low litter depth (0-5.0 cm). The sites were within 25 km of Fairbanks, in the areas of Goldstream Valley, the University of Alaska campus, and the Bonanza Creek Long-Term Ecological Research Forest. Sites ranged in latitude between 64°4250″ and 65°0617″N and in longitude between 147°2733″ and 148°1920″W (Table 4.1).

Measurements

In 2005 and 2006, we made week-long measurements (foliar insect and pathogen damage, sapflow, water potential, site climate) once per month at each site. At each site we measured the same eight alder genets that had been randomly selected in 2001 for the longer term study, which evaluated cumulative parasite damage (from foliar insect herbivore and pathogens) between 2001-2008 (Mulder and Roy, unpublished data). Our study focused all measurements on a single ramet within each of the eight marked genets. The primary consideration for including a ramet in our study was the suitability of the stem as an appropriate fit for the sapflow gauges (diameters 19 to 50 mm).

Damage survey

In 2005 and 2006, we visually estimated the percent leaf area damage by insect herbivores and pathogens during each month of the growing season (June, July, August) (Mulder et al. 2008). For each alder genet, foliar damage was estimated for the single ramet on which all measures of water balance measures were made. We evaluated leaves at three positions (inner, middle, and outer) within four branches representing the lower, middle, and highest parts of canopy (typically branch numbers 1, 4, 7, and 10) and recorded the different types of damage symptoms (i.e., shredding, central holes, linear holes, punctures, rasping).We then classified damage by insect feeding guild (chewing, mining, phloem feeding) or pathogen type (fungal, viral) (Mulder et al. 2008). Damage was expressed as the percent of leaf area damaged. If leaf area had been removed by herbivores, damage was expressed relative to the amount of leaf area originally present.

Leaf and sapwood area

During the pathogen and herbivore surveys, we measured leaf area in the field using a portable scanner CI-202 Area Meter (CID, Inc., Camas, Washington). In 2005, leaf area on the whole ramet was measured on leaves at three different leaf positions (inner, middle, outer) on the three branch positions (lower, middle, upper). In 2006, ramet leaf area was measured on every leaf that was surveyed for damage. We estimated canopy area for the ramet by counting the total number of leaves on each ramet and multiplying leaf number by the mean leaf area. The leaves that were surveyed in June and August of 2005 were collected, immediately weighed for fresh weight (to the nearest 0.0001 g), then dried at 55°C for 48 h. These measurements were used to calculate percent leaf moisture and specific leaf mass (g m^{-2}). The same dried leaves were then used for leaf chemistry measurements (%N, %C) to estimate the concentration of these elements in the beginning (June) and end (August) of the growing season. For each ramet, all the leaves were combined, ground on a roller mill (U.S. Stoneware, East Palestine, Ore.), and leaf chemistry was measured using a ThermoFinnigan Delta^{plus} XL (Thermo Fisher Scientific, Inc., Waltham, Mass.) with an ESC4010 elemental analyzer (Costech, Valencia, Calif.).

Live sapwood area was measured on alders (n=8 per site) from the same study sites. We were not able to make destructive measurements on the same alder ramets for which we measured sapflow, as they were part of the long-term monitoring study. Instead, we harvested different ramets (n=8 per site) within a similar size class to those

included in our study. Alder ramets were cut at the base and immersed in a 1 % aqueous safranin O solution (Ward's Natural Science, New York, NY) which stains live sapwood bright red. For every alder we stained, the entire diameter was composed of live sapwood with 1-3 millimeters of central pith and there was no heartwood formation. Since the pithy area represented a minor percentage of the total area stained bright red, we used basal area to approximate functional sapwood area.

Sapflow

In 2005, one ramet on each of eight alders was fitted with external stem flow gauges accommodating stem diameters ranging from 19 to 50 mm (SG-19, SG-25, SG-35, SG-50, Dynamax, Inc. Houston, TX., USA). In 2006, ramets of five alders were fitted with similar sized stem flow gauges. Sap flow was estimated using the Flow32 software package that calculates sap flow based on the heat balance method (Baker and van Bavel 1987). All gauges and adjacent portions of the stem were wrapped with foam insulation and then reflective foil to minimize temperature gradients caused by radiant heating of the stem. All gauges were operated without power to the heater for 24 hours to be certain that the foil and foam insulation shielded the stem from external temperature fluctuations (Gutierrez et al. 1994). A data logger (Model CR10x, Campbell Scientific Corp., Logan, UT., USA) continuously recorded mass flow of sap and means were logged every 15 minutes.

We characterized daily courses of sapflow using the parameters (slope, peak, and length) of diurnal sapflow curves, which indicate how sapflow responds to changes in

VPD. When daily courses of sapflow were compared across sites, sapflow was normalized by dividing by VPD. We also determined daily rates of water loss per ramet (J; g H_2O d^{-1}), normalized by leaf area (J_L; g H_2O m^{-2} leaf area d^{-1}) or by sapwood area (J_s ; g H_2O cm^{-2} d^{-1}) (Table 4.2). Each expression of water loss can reflect an influence of tree architecture on the physiological responsiveness to environmental variables (Meinzer et al. 2001). For example, water loss expressed on a leaf area basis is widely used for expressing rates of transpiration because the interception of energy is more closely related to leaf area (Kramer and Boyer 1995), while water loss expressed per unit of cross sectional sapwood area is an indication of whole plant water use (Meinzer et al. 2001).

Water potential

Midday (ψ_L) and predawn (ψ) water potential measures were made once per month (June, July, August) using a PMS pressure chamber (Albany, OR., USA) during the same week as sapflow measurements. Measurements were made on systematically selected branches at low, medium, and high parts of the canopy (branch numbers 1,4,7, and 10) on the same ramets that were fitted with sap flow gauges. On each branch, water potential was measured on a stem petiole bearing 2-4 leaves at a randomly chosen leaf position (inner, middle, outer) for a total of n=4 per alder. After all measurements were completed in August of 2006, we did an intensive sampling of inner and outer leaves on each of the selected branches to capture variation water potential differences from the inner to the outer parts of the branch on the selected ramet.

Hydraulic conductance

The apparent leaf area-specific hydraulic conductance of the soil/root/leaf pathway (G_t) was determined as:

$$G_t = J_L/\Delta\psi_L$$

where J_L is the mean transpiration rate per unit leaf area that was determined from sap flow measurements (Bucci et al. 2005). The difference between bulk soil ψ (determined from pre-dawn water potential measurements) and midday ψ_L is symbolized by ($\Delta\psi_L$). We confirmed that the pre-dawn measurements of ψ were taken when J_L approached zero, so that ψ was an adequate reflection of bulk soil ψ. G_t was calculated when midday values of ψ_L and J_L were relatively constant (Tausend et al. 2000).

Site Climate

At each site, we measured windspeed using a Vortex anemometer (Inspeed, Sudbury, MA with a pulse datalogger Madgetech, Warner, New Hampshire) that was vertically mounted on a pole at alder canopy height (2 m) in an open area around the center of the site. We also measured photosynthetically active radiation at three stations that were placed every 10 m along a transect that bisected the site. Each station had two level platforms (1.5 m in height) that held radiation sensors (LI-190 SA Quantum Sensor, LI-COR, Lincoln, Nebraska) connected to dataloggers (LI-1400 datalogger, LI-COR, Lincoln, Nebraska). Air temperature, relative humidity, and dew point were measured

(HOBO H8 Pro Series, Onset Comp. Corp., Bourne, MA.) at the central point of the site, 1.47 m above the ground surface.

During each measurement period at each site, four measurements of volumetric soil moisture (HH2 Moisture Meter, Delta-T Devices Ltd., England) and soil temperature at 10 cm (digital MULTI-thermometer, Infrared Thermometers.net) were made at the base of each alder. Moisture and temperature measurements were taken during the same time that midday and predawn water potential measures were made. In 2006, volumetric soil moisture was continuously logged at each site during the sapflow measurement period (SM200 soil moisture sensor, GP1 Data Logger, Delta-T Devices Ltd., England). Four sensors were installed at the sites using surface installation, which measured the top 50 mm of the soil. Since we monitored 5 alders at each site in 2006, one sensor was placed in between the two closest alders and the other 3 sensors were placed at the bases of the remaining 3 alders.

Data analysis

The alder ramets that we sampled from 2005-06 had a wide range of canopy areas (0.200 to 6.78 m^2) and a 10-fold range in the ratio of leaf area to sapwood area (LA/SA). To categorize this variability in plant architecture, we ranked the ramets according to LA/SA and divided the ranked distribution into three equal sections representing the upper range (0.706 to 0.349 m^2/cm^2 = high LA/SA), the middle range (0.339 to 0.177 m^2/cm^2 = medium LA/SA), and the lower range (0.177 to 0.046 m^2/cm^2 = low LA/SA). By dividing LA/SA into categories, we were able to use a repeated

measures approach to describe how monthly measurements of sapflow and leaf properties changed throughout the season. We used repeated measures ANOVA to explain seasonal differences in sapflow parameters (peak sapflow, initial slope, peak length) and total daily sapflow (J, J_L, J_s) across the LA/SA categories. The LA/SA groups were also used as categorical variables in a repeated measures ANOVA to explain seasonal differences in leaf properties (fresh and dry weight, specific leaf mass, % N, %C, C:N). After we used LA/SA as a categorical variable, we used LA/SA as a continuous variable in a regression to explain how J_L changed with LA/SA. We also used explained variation in water potential and hydraulic conductance (G_t) using LA/SA ratio and used regression to fit a polynomial function describing the relationship between LA/SA and G_t. Normality and homogeneity of variance of variables were checked before entry into the designated model and transformations were made as necessary. All analyses were performed with SAS (SAS Inst. version 9, Cary, N.C.).

RESULTS

Herbivore damage

In 2005, *A. fruticosa* experienced an early season defoliation event from chewing insects that resulted in the highest amount of total leaf damage (26.7 ± 1.6%) recorded during the 8 years that damage was monitored for *A. fruticosa* (Figure 4.1). Damage from other herbivores, including the phloem feeders (3.4 ± 0.4 %), miners (0.8 ± 0.1 %), and from pathogens (2.2 ± 0.3 %) during 2005 were all within ranges previously reported. In the years prior (2001-2004) and following (2006-2008) the defoliation event, cumulative

damage from herbivores and fungal pathogens did not exceed ~15 % (Mulder et al. 2008, Mulder and Roy unpublished data). Herbivores were not always present on the leaf so they could not be directly associated with the damage. However, the chewing insects that were present on alders include the Lepidopterans *Lophocampa maculata*, *Orygia antigua*, *Phlogophora* sp., and sawfly larvae (Hymenoptera) (Mulder et al. 2008).

The early season chewing damage resulted in variable amounts of leaf area loss. For example, our 2005 survey included heavily defoliated alders with 40- 83% damage, moderately defoliated alders with 20-30% damage, and alders with less than 5% chewing damage. In the following year, 2006, the cumulative amount of leaf area damage on alders was only 8.8 ± 0.6 % (Figure 4.1). Consequently, the alders that we sampled from 2005-06 had a wide range of canopy areas (0.200 to 6.78 m^2) and a 10-fold range in the ratio of leaf area to sapwood area (LA/SA) (Figure 4.2, Table 4.3).

LA/SA groups

Since herbivores remove leaf area, the LA/SA ratios are influenced by the amount of herbivore damage. However, the LA/SA ratio is also a reflection of the inherent variability of LA/SA. Consequently, ramets that ranked consecutively in the distribution did not necessarily have similar amounts of herbivore damage. For example, the majority of heavily damaged ramets were designated in the low LA/SA group; however, within this group a ramet with 17.39% damage (LA/SA of 0.134) ranked next to a ramet with 51.25% damage. Therefore, herbivore damage explains variation (R^2= 0.56) in the LA/SA groups ($F_{2,46}$=28.56, P = <0.0001). The remaining variation in LA/SA can be

attributed to other factors influencing plant architecture, such as the arrangement and position of the ramet within the genet and light availability within the entire genet canopy.

Leaf properties

Ramets from the low LA/SA group had consistently lower dry leaf mass throughout the season. However, the low LA/SA ramets had the greatest increase in specific leaf mass (SLM) from June to August, indicating that these alders built thicker leaves throughout the growing season (Table 4.4). Following defoliation, the low LA/SA alders also added the highest amount of nitrogen per unit leaf mass. The low LA/SA group increased leaf nitrogen per unit mass by 22%, while the middle and upper LA/SA groups increased leaf nitrogen per mass by only 5-6% (Table 4.4).

Sapflow parameters

We characterized daily patterns of water loss between the LA/SA groups using the parameters of diurnal sapflow curves. Peak flow reflects the maximum rate of daily water loss that is reached at the height of the diurnal sapflow curve. This parameter reflects the capacity for water loss per unit VPD (Peak) and per unit leaf area ($Peak_L$) (Table 3). Ramets in the low LA/SA group had significantly higher peak sap flow ($Peak_L$) relative to the other two groups (Table 4.5, Figure 4.3). In July, the mean peak sapflow ($Peak_L$) for the low LA/SA group was at least twice the rates reached in upper or middle LA/SA groups (Table 4.5). The greatest change in peak flow ($Peak_L$) was from July to August, when ramets in the low LA/SA group increased the rate of peak water loss by

53.7 %, while peak water loss decreased in the upper and middle LA/SA groups by 17.9 % and 40.4%, respectively (Table 4.5, Figure 4.3).

The slope parameter represents the initial slope of the diurnal sapflow curve, reflecting the increase in sapflow with increasing VPD. This parameter can reflect stomatal sensitivity to increasing VPD, as stomatal closure can limit the increasing rates of water loss as VPD approaches a midday high (Andrade et al. 2005). Alders in the lowest LA/SA group also had the highest water loss per unit increase in vapor pressure deficit (initial slope of the sapflow curve). For example, alders in the low LA/SA group lost 28 more grams of water per unit increase in VPD than the upper or middle LA/SA groups during August (Table 4.5). This indicates that alders in the low LA/SA group had less stomatal sensitivity to high VPD (Figure 4.3).

We also evaluated peak length, the period of time that maximum rates of sapflow (Peak flow) were maintained. This parameter can be an indication of stomatal regulation, as strong stomatal limitation of water loss can cause maximum rates of sapflow to remain nearly constant over a period of time despite increasing VPD (Andrade et al. 2005).The low LA/SA alders maintained peak flow over a consistent length of time (71 to 75 minutes). In contrast, alders in the upper and middle LA/SA groups transitioned to lower sapflow peaks across a longer period of time (Table 4.5). From July to August, alders in the upper and middle groups increased peak length by 43.9 minutes and 72.1 minutes, respectively.

Total daily water loss

Ramets in the low LA/SA group reached higher leaf area-based rates of daily sapflow (J_L) compared to the other LA/SA structural groups. June rates of J_L for the lowest LA/SA group (1474. 33 ± 124.53 g H_2O m^{-2} d^{-1}) were twice the June rates for the upper (643.64 g H_2O m^{-2} d^{-1}) and middle (702.33 g H_2O m^{-2} d^{-1}) LA/SA groups, which didn't differ (Table 4.6, Figure 4.4a). By July, J_L in low LA/SA alders (1495 g H_2O m^{-2} d^{-1}) was almost three times higher than alders in the high LA/SA group (563 g H_2O m^{-2} d^{-1}) and the medium LA/SA group (565 g H_2O m^{-2} d^{-1}) (Figure 4.4a). The low LA/SA group maintained the highest rate of J_L from July to August, as J_L declined in the medium LA/SA group by 237.12 g H_2O m^{-2} d^{-1} over these months.

Mean daily water loss expressed per ramet (J) was similar across the upper (1356.83 ± 244.43 g H_2O day^{-1}), middle (1121.08 ± 250.32 g H_2O day^{-1}), and lower (1359.62 ± 257. 00 g H_2O day^{-1}) LA/SA groups (Figure 4.4c). These results reflect the amount of ramet water loss without taking into account differences in plant architecture. However, mean daily sapflow per unit sapwood (J_s) was greatest in the highest LA/SA group (Figure 4.4b), reflecting the greater water demands associated with the larger canopy area in the high LA/SA group.

Rates of water loss expressed per ramet (J) and per unit sapwood area (J_s) changed through the season in the high and medium LA/SA groups. For the upper and middle LA/SA groups, J and J_s were highest during June and then declined from July to

August (Table 4.5, Figure 4.4). In contrast, all expressions of daily water loss (J_L, J, J_s) in the lowest LA/SA group were consistent throughout the season.

Herbivore damage in relation to daily water loss

Plant response to herbivore damage is traditionally compared across damage levels. However, we expected that total daily water loss (J_L) would be more related to LA/SA than the amount of leaf area damage. Herbivore damage and LA/SA can both be used to describe the variation in J_L (leaf area-based sapflow). In June, there was a positive linear dependence of J_L on herbivore damage ($R^2 = 0.36$; $P<0.0001$) (Fig. 4.5a). Although this linear relationship was maintained throughout the summer, herbivore damage explained less variation in J_L during July ($R^2=0.24$) and August ($R^2=0.16$). When plotted against LA/SA, J_L initially declined with increasing LA/SA and then gradually become level (Fig. 4.5b). Compared to herbivore damage, LA/SA described a higher amount of variation in J_L (Fig. 4.5a, b). J_L was best explained by LA/SA during July ($R^2 =0.43$; $P<0.0001$) (Fig 4.5b).

Site-level comparison

We expected that a decline in LA/SA would also have consequences for transpirational water loss under water limiting conditions; therefore, we evaluated sap flow across the LA/SA groups during a seasonal decline in site-level soil moisture. During the 2005-2006 summers, air temperature and precipitation were close to the long-term averages (1929-2007) for June and July (mean monthly temp.= 15.3 to 16.4 °C; mean monthly precip.= 34.5 to 50.0 mm) (www.climate.gi.alaska.edu/Climate).

However, August 2005 was warmer and drier (mean temp.= 14.1°C, mean precip. = 6.1 mm) than typical August weather (mean temp. = 13.2°C, mean precip. = 53.1 mm from 1929-2007).

This late-summer weather pattern in 2005 had different effects on the daily courses of sapflow for the low LA/SA alders at the two sites representing opposite ends of the local climate gradient. One site had strong seasonality in air and soil climate while the other site was moderate. The seasonal site had a more open overstory, which resulted in higher daily fluctuations in air climate. For example, in July, this site experienced higher levels of solar radiation that fuelled high air temperatures (15.8°C) and high evaporative demand (0.77± 0.02 kPa). This site also had cold night time temperatures (mean night temperature drop of 6.8 ± 0.1 °C) and a higher range of mean monthly air temperatures, which peaked in July (15.3 °C) but dropped to a low in August (9.0°C).

In contrast, the moderate site experienced a lower monthly range of air temperature (11.62 to 16.35°C). The moderate site also had the lowest day to night fluctuation of air temperature (3.83 ± 0.02 °C). Consequently, the seasonal site had drier soil in July (11% moisture) than the moderate site (22 % moisture). Ramets at the seasonal site also had the largest drop in midday water potential from July (-0.86 ± 0.06 MPa) to August (-1.24 ± 0.08 MPa).

In response to greater soil drying at the seasonal site, all LA/SA groups experienced midday depression in sapflow by August. However, ramets in the low LA/SA group consistently maintained higher rates of leaf area-based sap flow (July and

August) than other LA/SA groups at the site. Furthermore, low LA/SA alders steadily increased rates of water loss beyond the range of VPD at which other LA/SA groups experienced a midday depression in water loss. For example, the low LA/SA alders reached July peak flow at 1.84 kPa, which surpassed the VPD (1.6 kPa) at which medium LA/SA alders experienced a midday depression in sapflow (Figure 4.6a). As a result, the low LA/SA alders reached peak water loss (185.47 g H_2O hr^{-1} m^{-2}) when water loss had already declined to 118.87 g H_2O hr^{-1} m^{-2} in medium LA/SA alders. Alders in the high LA/SA group had the lowest rates of peak flow (73-74 g H_2O hr^{-1} m^{-2}) over the widest range of vapor pressure deficit (1.5 to 1.8 kPa).

By August, low LA/SA alders at the seasonal site reached peak water loss of 200 g H_2O $hr^{-1}m^{-2}$, similar to the rates in June (184 g H_2O hr^{-1} m^{-2}) and July (185 g H_2O hr^{-1} m^{-2}). The low LA/SA group experienced a midday depression in August sapflow near 1.23 to 1.3 kPa and rates of water loss continued to decline with increasing VPD (Figure 4.6b). However, following the midday depression, the low LA/SA alders still had the highest rates of sapflow compared to the other LA/SA groups and reached a second, lower sapflow peak (146.23 g H_2O hr^{-1} m^{-2}) near the daily high in VPD (1.63 kPa). The medium LA/SA group reached peak flow of 53-59 g H_2O hr^{-1} m^{-2} from 1.3 to 1.61 kPa, while the high LA/SA group reached peak flow of 101-88.6 g H_2O hr^{-1} m^{-2} over a range of 1.04 to 1.53 kPa (Figure 4.6b).

At the moderate site, low LA/SA alders had consistently higher rates of water loss than the other LA/SA groups. In June, the low LA/SA alders reached peak water loss

(100 g H_2O hr^{-1} m^{-2}) between 2.1 to 2.4 kPa, which exceeded the VPD (2.2 kPa) at which high LA/SA alders experienced a midday depression in sapflow (not shown). In July, the low LA/SA alders reached peak water loss (167.74 g H_2O m^{-2} hr^{-1}) during the midday high in VPD (2.47 kPa). Medium LA/SA alders experienced a midday depression in sapflow after reaching peak flow (76.3 g H_2O hr^{-1} m^{-2}) at 2.14 kPa, but fully recovered later in the day reaching a second sapflow peak (81.62 g H_2O hr^{-1} m^{-2}) at 2.47 kPa (Figure 4.7a). The largest difference between the LA/SA groups was in August, when peak flow rates in the low LA/SA alders were the highest for the season (175.22 to 184.31 g H_2O hr^{-1} m^{-2}). During August, low rates of peak water loss were maintained in the highest LA/SA group (40.89 to 51.27 g H_2O hr^{-1} m^{-2}) and medium LA/SA group (18.6 to 31.22 g H_2O hr^{-1} m^{-2}) over a wider range of VPD (1.49 to 2.25 kPa) (Figure 4.7b). In contrast to August sapflow at the seasonal site, the lowest LA/SA group at the moderate site did not experience a midday depression in sapflow by August.

Water potential and liquid phase hydraulic conductance

Despite the differences in rates of leaf-area based water loss, the three LA/SA groups maintained similar levels of mean midday water potential in July and August (-0.93 to -1.11 MPa). The majority of midday water potential measurements remained above -1.2 MPa (Figure 4.8a). Predawn water potential values were also similar for all LA/SA groups across the season and did not drop below -0.5 MPa.

Since alders maintained leaf water balance with increased transpiration rates, we also expected to find an accompanied increase in liquid phase hydraulic conductance. We

calculated liquid phase hydraulic conductance (G_t) of the soil/leaf pathway as the ratio of leaf area-based transpiration rate (J_L) to the driving force for sapflow (the pressure difference between the leaves and the soil). G_t was negatively correlated with LA/SA, and the relationship was significantly described by a polynomial function (Figure 4.8b). High values of G_t (above 400 g H_2O hr^{-1} m^{-2} MPa^{-1}) were maintained up to an LA/SA of 0.2 m^2/cm^2, after which G_t declined and leveled at values < 200 g H_2O hr^{-1} m^{-2} MPa^{-1}.

DISCUSSION

Enhanced water relations at lower LA/SA

Our results confirm our first hypothesis that ramets with higher herbivory and lower LA/SA support enhanced rates of leaf area-based water loss. Diurnal sapflow curves indicate that low LA/SA alders had the highest peak rates of leaf area-based water loss during the daily maximum driving conditions (VPD) and higher water loss per unit increase in VPD (initial slope of the sapflow curve). Furthermore, low LA/SA ramets sustained peak sapflow rates for surprisingly consistent lengths of time (peak length) throughout the growing season. This enhanced performance over the daily course of sapflow translated to higher rates of daily water loss (J_L, leaf area-based) in the low LA/SA alders compared to similar J_L in the lower and the middle LA/SA groups. We expected the low LA/SA alders to support higher rates of leaf area-based water loss, since a lower LA/SA indicates a higher capacity for water transport (sapwood area) relative to the potential water demand (leaf area) (Sala et al. 2001, Choat et al. 2007). Interestingly, the low LA/SA alders maintained enhanced water loss even during the first measurement

period (June), when the heavy chewing damage took place. This suggests that alders may have quickly sealed off wounds from herbivore damage. Plants have been shown to lignify the edges of wounded leaves and re-gain control over water loss within one week following herbivore damage (Aldea et al. 2005).

Herbivore damage can be used to describe variation in transpirational water loss per unit leaf area (J_L) (Fig. 4.5). However, the post-herbivory LA/SA ratio consistently explained more variation in J_L and J_S in June, July, and August. Furthermore, as a morphological index, LA/SA is a functionally meaningful index that will be coordinated with other plant functions. For example, a similar relationship between LA/SA and J_L has been shown in other studies that have also measured coordinated increases in stomatal conductance and leaf-area specific total hydraulic conductance (G_t) with declining LA/SA (Tausend et al. 2000, Andrade et al. 2005). Using the LA/SA ratio can also be useful for evaluating other types of pest damage that could impose hydraulic limitations on the plant. For example, the Cytospora canker disease on alders kills vascular tissue and blocks water transport within the stem (Rohrs-Richey et al. 2010). Disease-related reduction in functional sapwood area would certainly reduce water supply to the leaves and the ability of the plant to support a full canopy. Therefore, LA/SA is a functionally useful morphological index for considering the effects of both types of pest damage on leaf area based water transport.

Site level comparison of LA/SA groups

We expected that the decline in LA/SA would also have consequences for transpirational water loss under water limiting conditions. For example, with a higher amount of sapwood area relative to leaf area, the low LA/SA plants can supply enough water to the canopy to prevent the development of damaging water potential gradients (McDowell et al. 2002) while limiting the degree of stomatal closure (Sala et al. 2001). With adequate water supply to the canopy, it is likely that the low LA/SA alders avoided extensive stomatal closure, allowing higher rates of water loss and carbon uptake during daily highs in VPD. Water loss was more restricted in the upper and middle LA/SA groups, suggesting that these groups used stomatal regulation during daily highs in VPD to prevent the development of high xylem tensions (McCulloh and Sperry 2005). This also suggests that the upper and middle LA/SA groups may be more susceptible to water stress (Sala et al. 2001).

We evaluated this more closely at a site (the "seasonal site") that had strong fluctuations in air and soil climate and experienced the greatest amount of soil drying during late summer 2005. As we expected, daily courses of sapflow at the seasonal site indicate that even during drier conditions, ramets with lower LA/SA maintained higher rates of water loss beyond the range of VPD at which the other LA/SA groups experienced midday depression in sapflow (Figure 4.6).

The drier soil conditions affected all LA/SA groups at the seasonal site, and the low LA/SA group eventually used stomatal regulation to restrict midday water loss in

August. However, the VPD at which this midday depression occurred was higher for the low LA/SA group compared to the other two groups. For example, the July daily courses of sapflow at the seasonal site indicate that the high LA/SA alders experienced midday depression at 1.6 kPa, while rates of water loss continued to increase in the low LA/SA alders until 1.9 kPa. With more transpiring surface area to supply with water, the alders with higher LA/SA ratios had to restrict water loss at a lower VPD. This result indicates that alders with a higher LA/SA ratio are more susceptible to dangerous xylem tensions at lower VPDs, which suggests that these alders will have greater stomatal sensitivity to high VPDs. In order to prevent hydraulic failure under water-limitation, alders with higher LA/SA ratios will restrict water loss at a lower VPD than alders with low LA/SA.

Coordinated mechanisms with lower LA/SA

The successful regulation of water loss can be indicated by the maintenance of leaf water potential. Despite different rates of water loss among the LA/SA groups, we found that mean values of midday water potential were maintained above -1.1 MPa in all groups, which is just above the pressure (-1.2 MPa) at which *A. fruticosa* experience 50% embolism (or 50% loss in hydraulic conductivity) (Sperry et al. 1994). We did not measure any midday water potentials near -2.0 MPa, which has been associated with 70-80% loss of hydraulic conductivity (Sperry et al. 1994). The water potential measures in this field study suggests that alders use stomatal control of water loss to maintain homeostasis of leaf water balance (Meinzer and Grantz 1990) and indicate that alders

maintain leaf water potential at or above a threshold level (Bond and Kavanagh 1999, Oren et al. 1999).

Since alders in the low LA/SA group maintained leaf water potential with increased transpiration rates per unit leaf area, we also expected to find an accompanied increase in liquid phase hydraulic conductance (G_t) of the soil/leaf pathway. We found the highest values of G_t at lower values of LA/SA (under 0.2 m^2/cm^2), and a similar relationship has previously been determined in other studies relating G_t and LA/SA (Tausend et al. 2000, Bucci et al. 2005). G_t has also been shown to be directly related to increases in leaf-specific conductivity (ratio of flow rate across the leaf to the driving force for flow) and increased stomatal conductance (g_s) (Meinzer and Grantz 1990, Pataki et al. 1998, Tausend et al. 2000). Plants with lower LA/SA theoretically have higher leaf-specific conductivity because of the increased capacity of the vascular system to supply water to the leaves (Choat et al. 2007). Consequently, the higher leaf-specific conductivity can assist in maintaining xylem water potentials above a level that would trigger drought-induced embolism during low water availability (Choat et al. 2007). It is likely that the coordination of these mechanisms (G_t, g_s, leaf-specific conductivity) allowed low LA/SA alders to achieve the highest rates of leaf area-based water loss while also maintaining homeostasis of leaf water balance (Meinzer and Grantz 1990, Meinzer et al. 2004).

Enhanced rates of water loss after defoliation or canopy thinning can also be attributed to an improvement of the microclimate for the remaining foliage. If the canopy

is sufficiently closed prior to herbivory, reductions in leaf area can increase light penetration and wind exposure, which can lead to greater stomatal conductance and transpiration (Pataki et al. 1998, Oren et al. 1999). Although this may have occurred to some extent in our study, the fact that low LA/SA alders maintained consistent rates of total daily water loss across a wide range of seasonal and environmental conditions indicates that sapflow was primarily regulated by the internal, coordinated mechanisms (G_t, g_s, leaf-specific conductivity) that adjusted to the herbivory-related shifts in LA/SA.

Implications for carbon gain and assimilation

It is likely that enhanced rates of water loss were also related to higher rates of carbon uptake and assimilation in the low LA/SA group. The higher G_t in low LA/SA ramets was likely correlated with higher rates of carbon assimilation. There is a positive linear association between G_t, g_s, and carbon assimilation that reflects the close link between hydraulic transport and photosynthetic capacity at the whole plant level as well as the control and coordination of internal physiology at the leaf level (Franks and Brodribb 2005). Although we only calculated G_t at the ramet-level, the potential for coordination of higher rates of transpiration, stomatal conductance and photosynthesis has been previously shown at the leaf-level for *A. fruticosa* (Rohrs-Richey et al. 2010). Therefore, it is likely that herbivory-related declines in the LA/SA ratio were followed by whole plant adjustments in G_t and corresponding leaf-level adjustments in g_s and carbon assimilation.

The increased potential for carbon gain was also reflected by leaf properties in low LA/SA group. Following defoliation, the low LA/SA alders added the highest amount of nitrogen per unit leaf mass. The low LA/SA group increased leaf nitrogen per unit mass by 22%, while the middle and upper LA/SA groups increased leaf N per mass by only 5-6%. This suggests that the low LA/SA alders increased the amount of photosynthetic machinery per unit leaf mass to offset the loss of leaf area. Alternatively, N resorption may have been delayed in the low LA/SA group.

Implications for tolerance and compensation

One way to gauge the success of the compensatory response we measured is by linking compensation to fitness through measures of tolerance. Evolutionary ecologists define tolerance as a reaction norm of fitness, measured as the slope of a regression of fitness on disease severity for each host genotype (Simms 2001, Schürch and Roy 2004). For example, a completely tolerant genotype has a flat reaction norm (slope = 0) and experiences no fitness impact of damage (Stowe et al. 2000). Measures of tolerance traditionally compare responses in damaged relative to undamaged plants. However, our study shows that defining damage levels relative to LA/SA is a more functionally relevant approach to assessing tolerance to defoliation in *A. fruticosa.*

However, our results raise the question: are adjustments in transpirational water loss are an appropriate measure of tolerance to herbivory? In evolutionary terms, tolerance relies on the assumption that the response to herbivory is governed by a specific adaptation to consumer damage. The response that we measured may have instead been a

result of a general adaptation to stress or competition within the evolutionary constraints of water transport. This is consistent with the idea that rather than being a specific adaptation to consumer damage, tolerance of herbivore damage is a by-product of selection for the ability to tolerate other environmental stresses (Stowe et al. 2000). As an index of hydraulic architecture, it seems more plausible that LA/SA is related to series of underlying traits that could also confer tolerance to specific consumer damage. While our study cannot resolve which traits may confer tolerance *to* herbivory, our study does have implications for the capacity to compensate *for* herbivory. Our study suggests that evaluating herbivore damage in relation to the LA/SA ratio can increase the predictability of the impact of herbivores on plant function.

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Table 4.1 Site information. Sites were partly selected based on mean summer air temperatures in 2003-2004. All sites had a narrow range of minimum winter temperatures in 2004 (-27.35 to -29.31 °C from December to February).

Site	Lat (N)	Long (W)	Elev (m)	Summer Air Temp. (°C)[1]
Univ. of AK.	64° 5147″	147°51 39″	140	5.22 ± 0.08
Goldstream Valley	65°06 17″	147°27 33″	290	6.69 ± 0.08
Bonanza West	64°42'51″	148°19 21″	220	9.98± 0.01
Bonanza East	64°4481″	148°18 45″	355	10.11± 0.01
Bonanza South	64°4456″	148°18 77″	360	8.34± 0.08
Cripple Creek	64°46 42″	148°01 50″	180	9.23 ± 0.01
Univ. of AK. South	64°5147″	147°51 39″	180	13.15± 0.01

Table 4.2 Definitions of symbols and parameters.

Symbol or parameter	Definition
LA/SA	Leaf area: sapwood area (m^2/cm^2)
VPD	Vapor pressure deficit (kPa)
J_L	Leaf area-based total daily water loss (g H_2O m^{-2} d^{-1})
J	Total daily water loss per ramet (g H_2O d^{-1})
J_s	Total daily water loss per unit sapwood area (g H_2O cm^{-2} d^{-1})
ψ_L	Midday water potential (MPa)
Ψ	Pre-dawn water potential (MPa)
G_t	Apparent leaf area-specific hydraulic conductance of the soil/root/leaf pathway (g H_2O hr^{-1} m^{-2} MPa^{-1})
Peak	Maximum rate of daily water loss that is reached at the height of the diurnal sapflow curve (g H_2O hr^{-1} kPa^{-1})
$Peak_L$	Maximum rate of leaf area-based daily water loss that is reached at the height of the diurnal sapflow curve (g H_2O hr^{-1} m^{-2} kPa^{-1})
Slope	Initial slope of the diurnal sapflow curve, reflecting the increase in sapflow with increasing VPD (g H_2O hr^{-1} m^{-2} kPa^{-1})
Peak length	Period of time that maximum rates of sapflow were maintained (minutes)

Table 4.3 Leaf and canopy area across LA/SA structural groups. Damage is reported as % leaf area. "Sap. area" is sapwood area. Tests for differences between means based on the Tukey-Kramer adjustment in the GLM procedure. Significant differences between LA/SA groups at the α=0.05 level are indicated by letters; NS = not significant.

Month		High LA/SA	Medium LA/SA	Low LA/SA	F-value	P-value
June						
	Damage (%)	8.34 ± 1.67**a**	2.43 ± 0.55**a**	30.53 ± 6.07**b**	6.62	0.003
	Canopy area (m^2)	2.69 ± 0.43**a**	2.18 ± 0.30**a**	1.08 ± 0.21**b**	10.66	0.0002
	Sap. area (m^2)	0.0008±.0002	0.0011±.0003	0.0011±.0002	3.68	NS
	Leaf area (cm^2)	38.84 ± 2.62**a**	33.55 ± 2.70**a**	23.52 ± 1.74**b**	10.22	0.0002
	Leaf number	730 ± 135	632 ± 117	560 ± 79	1.20	NS
	LA/SA (m^2/cm^2)	0.455 ± 0.03**a**	0.27 ± 0.01**b**	0.11 ± 0.01**c**	84.56	0.0001
Aug						
	Damage (%)	13.97 ± 2.89	7.79 ± 1.15**a**	26.18 ± 6.53**b**	4.74	0.014
	Canopy area(m^2)	2.35 ± 0.43**a**	2.07 ± 0.34**a**	1.00 ± 0.19**b**	7.30	0.002
	Leaf area(cm^2)	31.86 ± 2.81**a**	31.11 ± 4.97	21.50 ± 2.56**b**	3.78	0.036
	Leaf number	826 ± 186	871 ± 266	523 ± 99	2.44	NS
	LA/SA (m^2/cm^2)	0.34 ± 0.04**a**	0.24 ± 0.03**a**	0.11 ± 0.01**b**	18.86	<.0001

Table 4.4 Leaf properties and characteristics across LA/SA groups. Significant differences between LA/SA groups at the α=0.05 level are indicated by letters. Repeated measures tests were used to detect significant effects of time and the interaction between time and LA/SA group. For fresh and dry mass, SLM, denominator (den.) d.f. = 77. For % N, %C, C:N, den. d.f. = 36. These tests use the Greenhouse-Geisser Epsilon Adjustment to adjust degrees of freedom for within subject tests. Significance level for both univariate and repeated measures tests; *** P< 0.0001; **P<0.001; *P<0.05; NS = not significant.

Property	Month	High LA/SA	Medium LA/SA	Low LA/SA	Denom. d.f.	Repeat. Measure
Fresh mass (g)	June	0.52 ± 0.05 **a**	0.51± 0.04 **a**	0.33 ± 0.02**b**	83	NS
	Aug	0.48 ± 0.03 **a**	0.46 ± 0.04	0.37 ± 0.03**b**		
Dry mass (g)	June	0.22 ± 0.02 **a**	0.21 ± 0.03	0.14 ± 0.01**b**	78	NS
	Aug	0.21 ± 0.14 **a**	0.22 ± 0.02 **a**	0.16 ± 0.01**b**		
SLM ($g\ m^{-2}$)	June	57.86 ± 4.28	62.15 ± 6.28	63.35 ± 3.71	77	NS
	Aug	57.26 ± 2.07 **a**	62.48 ± 2.89	68.77 ± 1.89**b**		
N (%)	June	1.62 ± 0.03	1.56 ± 0.07	1.62 ± 0.05	36	Time*** Inter**
	Aug	1.86 ± 0.04	2.16 ± 0.04	2.09 ± 0.04		
C (%)	June	39.06 ± 0.46	39.45 ± 1.05	40.00 ± 0.54	36	NS
	Aug	39.16 ± 0.53	37.31 ± 0.33**a**	35.89 ± 0.28**b**		
C:N	June	24.71 ± 0.57	25.74 ± 1.07	25.61 ± 0.99	36	Time***
	Aug	19.44 ± 0.74	17.42 ± 0.34	17.69 ± 0.89		
N per mass ($mg\ g^{-1}$)	June	3.94 ± 0.40 **a**	3.61 ± 0.55 **a**	2.04 ± 0.21 **b**	16	Time*** Inter***
	Aug	4.16 ± 1.03	3.80 ± 0.37 **a**	2.63 ± 0.28 **b**		
N per area ($\mu g\ cm^{-2}$)	June	98.66 ± 7.64	96.28 ± 9.68	92.04 ± 4.97	17	Time***
	Aug	130.18 ± 7.24	133.33 ± 7.27	135.94 ± 6.31		

Table 4.5 Sapflow parameters for each LA/SA group. Peak sapflow is reported as normalized by VPD (Peak) and also per unit leaf area ($Peak_L$). The parameter "slope" represents the initial slope of the sapflow curve and reflects the amount of water lost per unit increase in VPD. Peak length reflects the amount of time that peak sapflow is maintained. The last two columns of the table represent the results of repeated measures analysis and list the effect of LA/SA (Group) and the effect of the measurement period (Time) on the sapflow parameter. Denominator degrees of freedom are as follows: peak d.f=30, peak length d.f = 25, slope d.f.=18. These tests use the Greenhouse-Geisser Epsilon Adjustment to adjust degrees of freedom for within subject tests. Where the effect of time was detected, only the July to August time contrasts were significant. NS=not significant.

Parameter	Month	High LA/SA	Medium LA/SA	Low LA/SA	Repeated Measures	
					Group Effect	Time Effect
Peak ($g\ H_2O\ hr^{-1}\ kPa^{-1}$)	June	137.54 ± 24.57	161.81 ± 47.96	91.42 ± 14.00	P=0.026	NS
	July	161.45 ± 26.65	176.78 ± 47.96	136.83 ± 23.29		
	August	136.41 ± 19.99	119.52 ± 29.45	217.26 ± 53.22		
$Peak_L$ ($g\ H_2O\ hr^{-1}\ m^{-2}\ kPa^{-1}$)	June	54.20 ± 9.07	71.26 ± 13.17	99.72 ± 10.62	P=0.010	NS
	July	77.29 ± 8.86	82.94 ± 17.61	162.35 ± 25.03		
	August	63.41 ± 8.74	49.45 ± 9.01	249.82 ± 50.77		
Slope ($g\ H_2O\ hr^{-1}\ m^{-2}\ kPa^{-1}$)	June	52.82 ± 9.27	64.58 ± 10.15	58.31 ± 7.20	NS	P=0.028
	July	56.83 ± 9.50	71.22 ± 14.51	83.62 ± 11.84		
	August	43.37 ± 7.23	42.16 ± 11.52	71.84 ± 18.55		
Peak length (minutes)	June	45 ± 7.7	83.08 ± 19.26	71 ± 17.23	NS	P=0.032
	July	75 ± 22.34	50.63 ± 15.51	75 ± 18.45		
	August	118.93 ± 38.45	122.73 ± 28.30	75 ± 20.97		

Table 4.6 Effect of time and LA/SA group on total daily water loss. Repeated measures tests use Roy's Greatest Root with 2 numerator degrees of freedom and 31 denominator degrees of freedom. The Greenhouse-Geisser Epsilon Adjustment was used to adjust degrees of freedom for within subject tests. When a time effect was detected, only the July to August time contrast was significant. There were no significant interactions between LA/SA group and time.

Effect	Sapflow, J_L ($g\ H_2O\ m^{-2}\ d^{-1}$)	Sapflow, J ($g\ H_2O\ cm^{-2}$)	Total water use, J_s ($g\ H_2O\ cm^{-2}\ d^{-1}$)
LA/SA group	$F_{2,32}$= 22.67	---	$F_{2,32}$= 4.01
	P=<.0001		P=0.0279
Time	---	$F_{2,64}$= 22.00	$F_{2,64}$= 12.35
		P=<.0001	P=<.0001

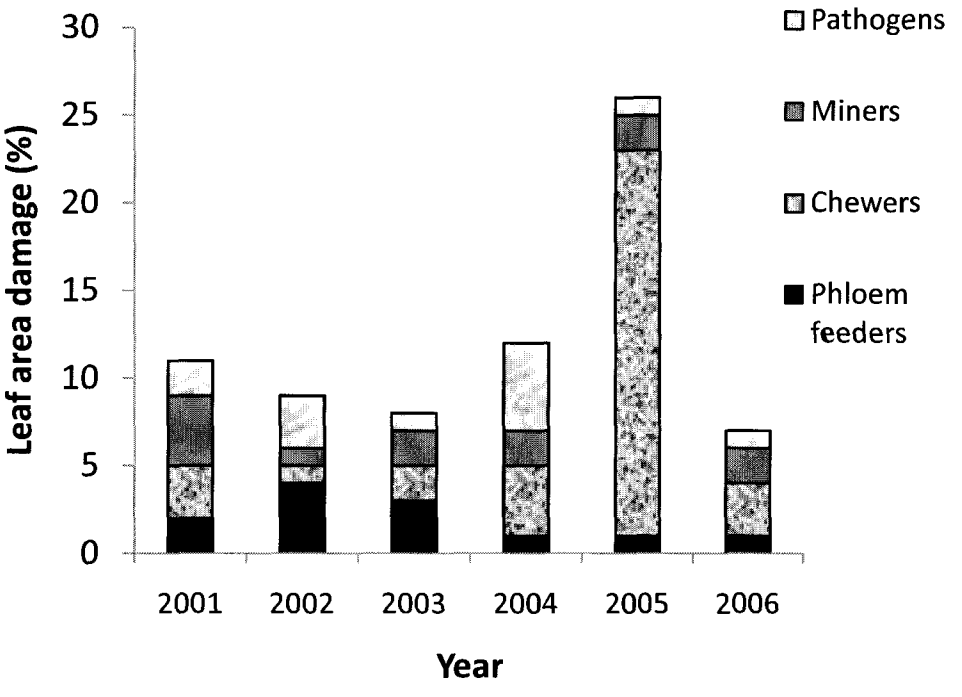

Figure 4.1 Leaf area damage from different feeding guilds. This illustration reflects reported damage estimates from Mulder et al. 2008.

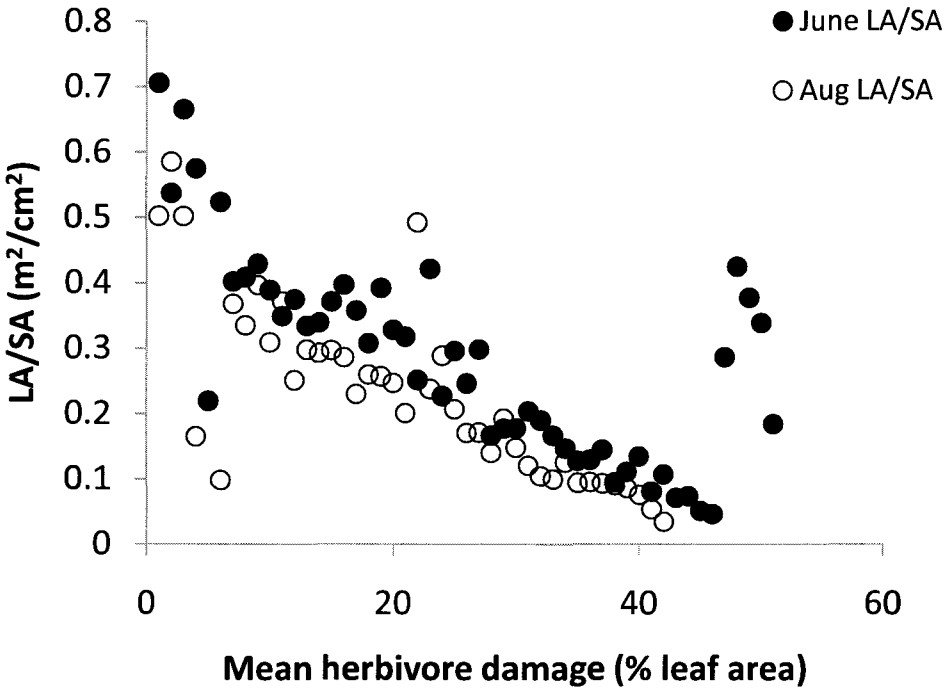

Figure 4.2 LA/SA vs. leaf area damage. The relationship between ranked LA/SA data and mean herbivore damage for the months of June (●) and August (○). This graph is the basis for the division of alders into the three LA/SA groups. Each data point represents one alder. Standard errors associated with each point are not shown to maintain the clarity of the graph. Standard errors associated with mean herbivore damage (based on % leaf area) are: Group 1=1.67%, Group 2=0.55%, and Group 3=6.07%. Standard errors associated with LA/SA were similar for all groups (0.002 to 0.003 cm^2/m^2).

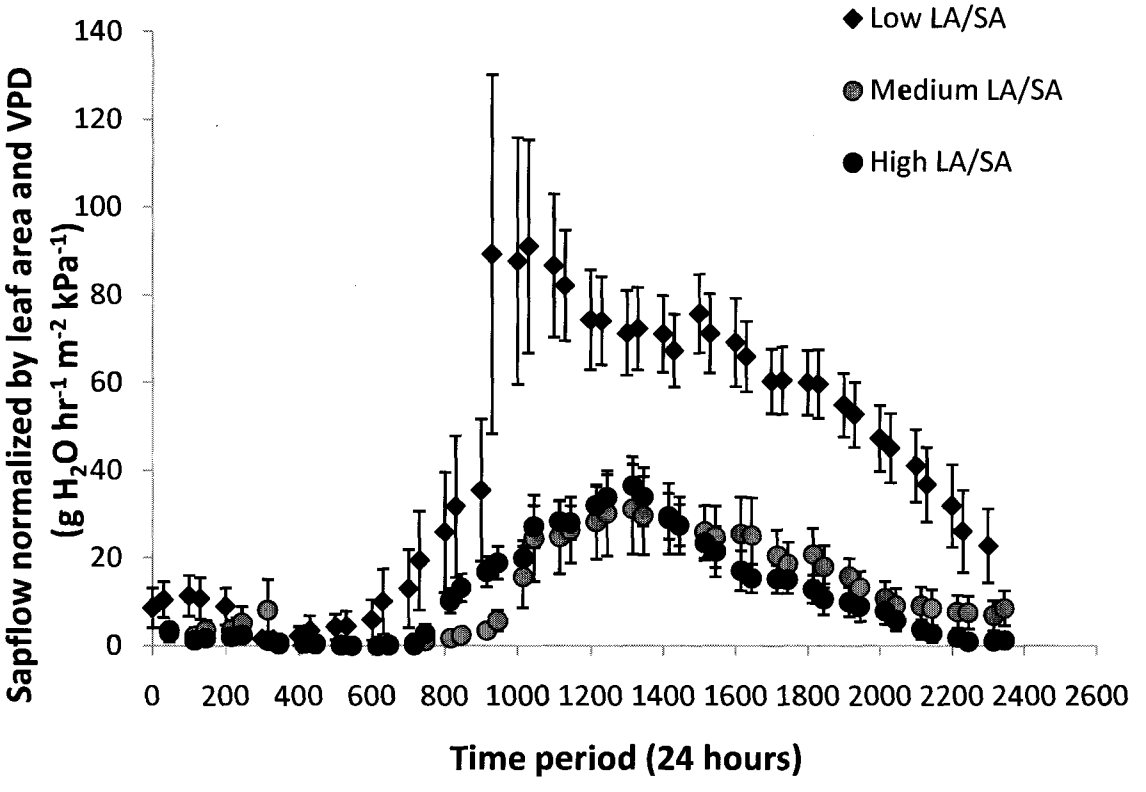

Figure 4.3 August daily sapflow for all LA/SA groups. Differences in daily rates of sapflow (normalized by leaf area and VPD) are shown for a 24-hour period for each LA/SA group: high (●), medium (grey ○), and low (♦) LA/SA. Data are based on the maximum sapflow rates measured during the 5-day rotational measurement period.

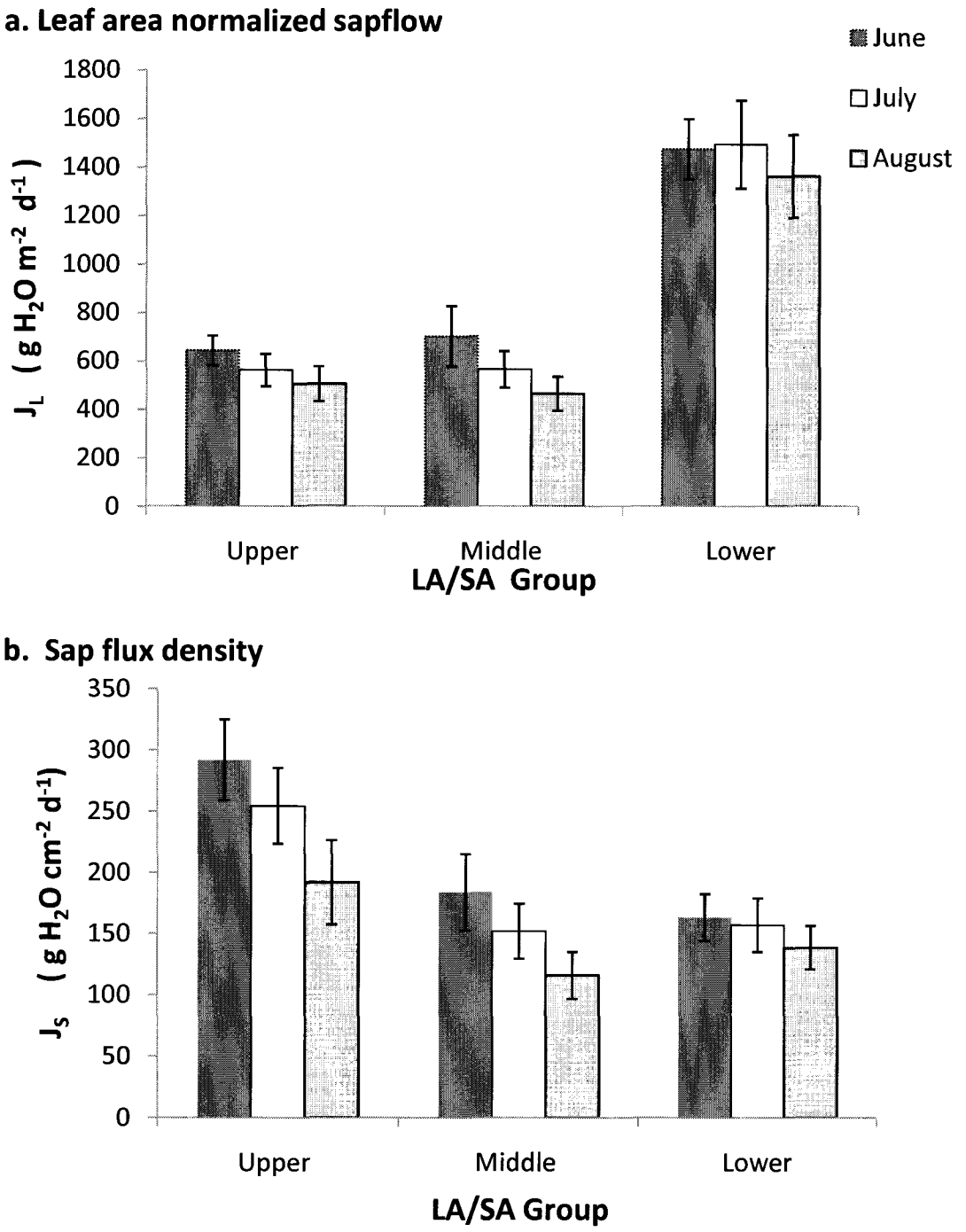

Figure 4.4 Total daily sapflow across the growing season. a) Leaf-normalized daily sapflow (J_L); means ± 1 S.E. in each LA/SA group for June (solid), July (no color), and August (stippled). b). Sapwood area normalized sapflow (J_s); means ± 1 S.E. c) Sapflow per ramet (J); means ± 1 S.E. All reported means are based on the days of maximum sapflow rates for each 5 day measurement period. Repeated measures statistics for this graph are listed in Table 5.

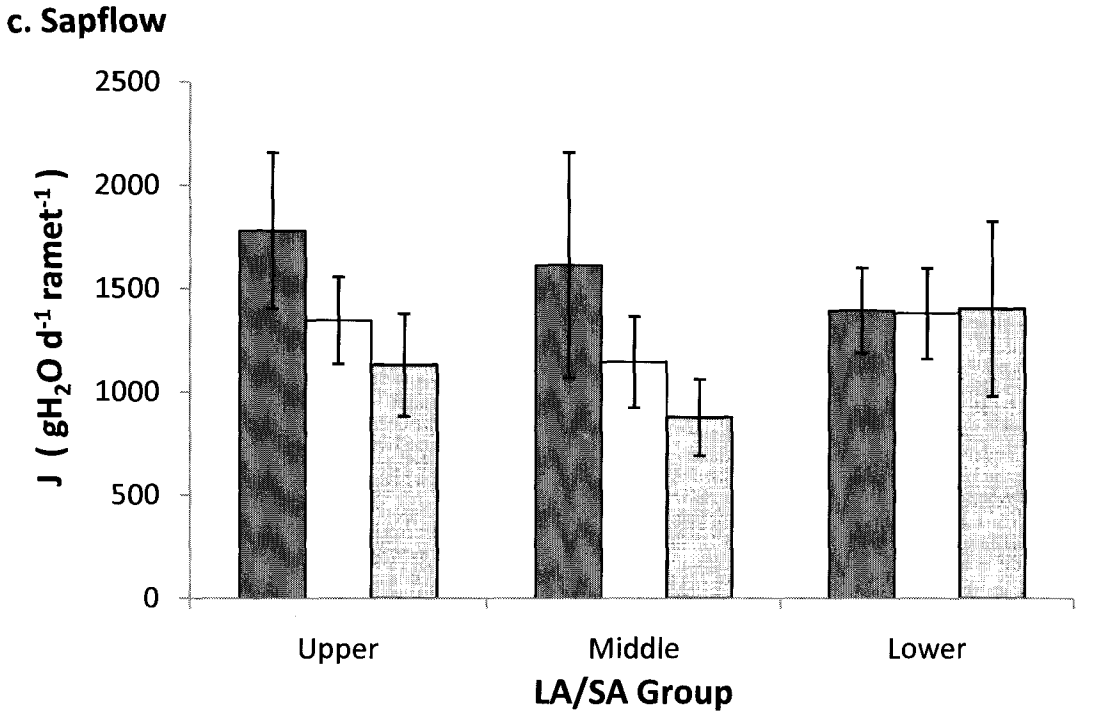

Figure 4.4 continued.

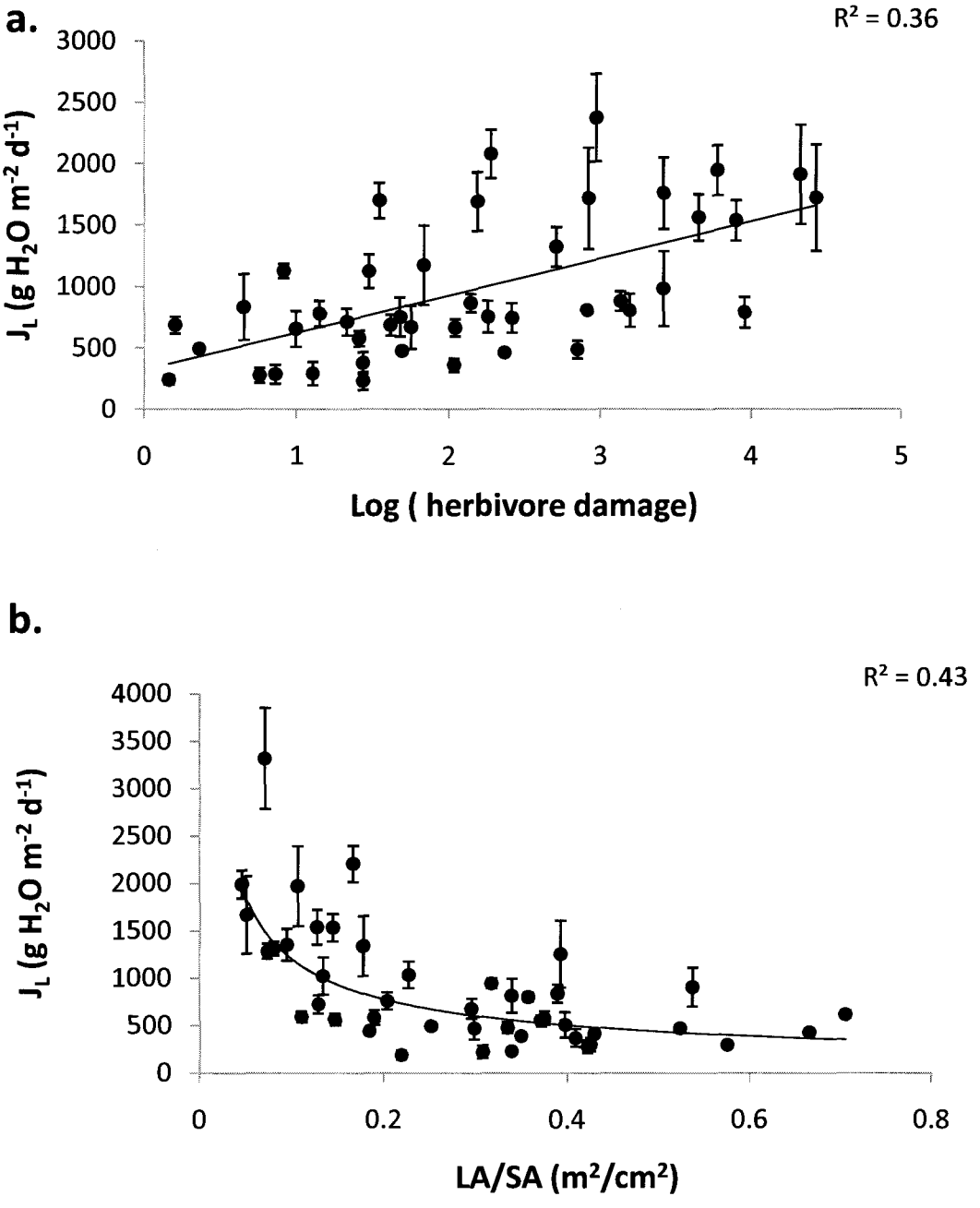

Figure 4.5 J_L in relation to damage and LA/SA. a) The relationship between J_L and damage is shown for June, when damage described the highest amount of variation in J_L ($P<.0001$). By August, herbivore damage explained only 16% of the variation in J_L. b) The relationship between J_L and LA/SA is shown for July; $J_{LJuly} = 280.94\ (LA/SA)^{-0.631}$. Means are based on the days of maximum sapflow rates for each 5 day measurement period.

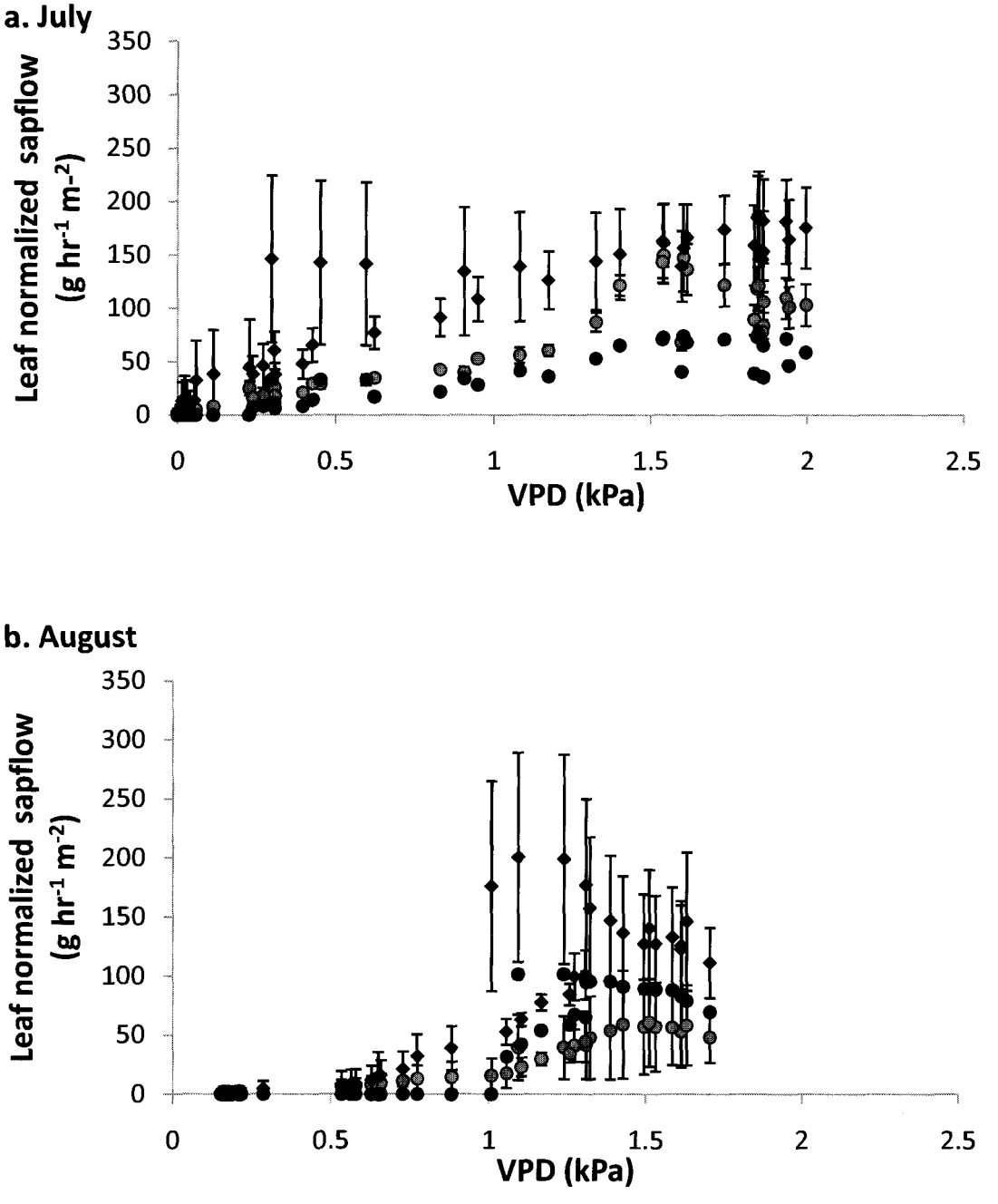

Figure 4.6 Daily sapflow at the seasonal site. Half-hourly means ± 1 S.E. of daily sapflow for the high (●), medium (grey ○), and low (♦) LA/SA groups shown for select days in (a) July and (b) August. June is not shown because LA/SA groups had similar courses of sapflow during that month. For July (b), sapflow data was valid for only 1 alder in each of the two groups. Days were selected based on the consistency of sapflow data for alders in all three LA/SA groups.

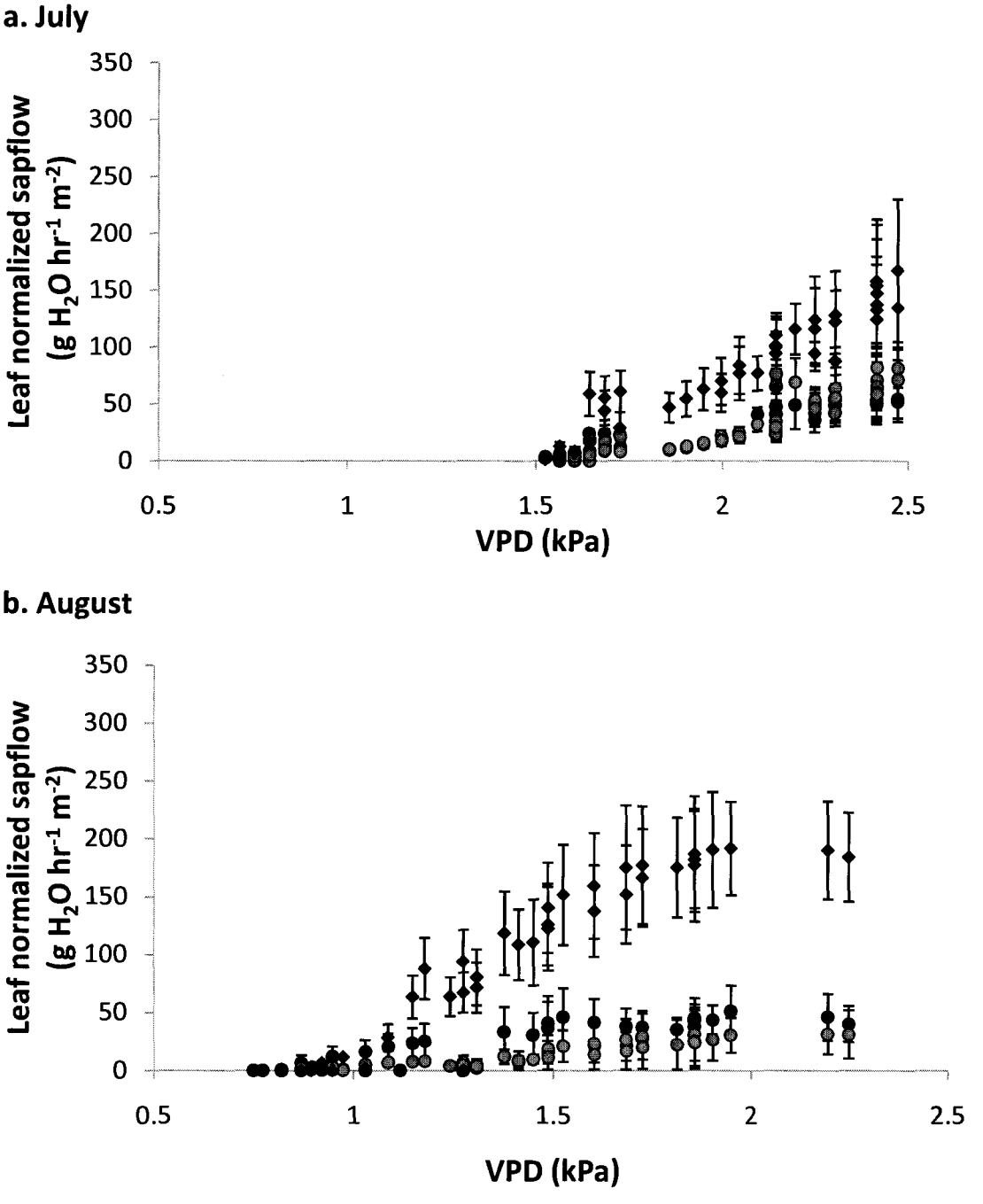

Figure 4.7 Daily sapflow at the moderate site. Half-hourly means ± 1 S.E. of daily sapflow in the high (●), medium (grey ○), and low (♦) LA/SA groups shown for select days in (a) July and (b) August. June is not shown because LA/SA groups had similar courses of sapflow during that month. Days were selected based on the consistency of sapflow data for alders in all three LA/SA groups.

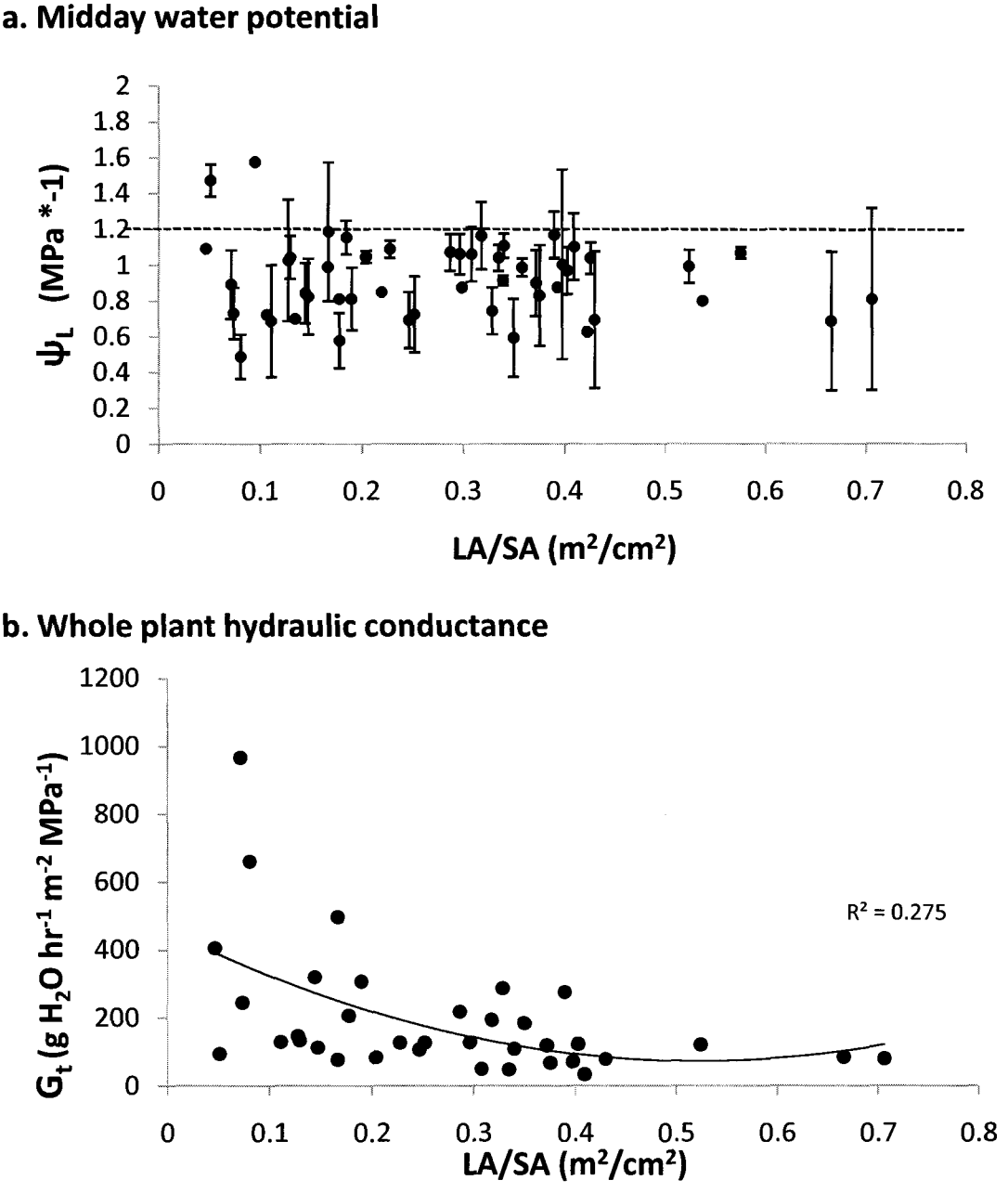

Figure 4.8 Water potential and hydraulic conductance across LA/SA. (a) Each point reflects the seasonal midday water potential (ψ_L) means ± 1 S.E for individual alders (n= 52). The stippled line indicates the water potential (-1.2 Mpa) at which green alders experience 50% embolism (or 50% loss in hydraulic conductivity) (Sperry et al. 1994). (b) Whole plant liquid phase hydraulic conductance (G_t). Each point represents the seasonal mean ± 1 S.E for individual alders (n= 35). A polynomial function (y= 1417.7 x^2 – 1477.4x + 457.15; R^2= 0.275; P=0.005) describes the relationship between LA/SA and G_t.

CHAPTER 5

GENERAL CONCLUSIONS

The overall aim of my work was to evaluate the response of *A. fruticosa* to two types of biotic pest damage. My approached evaluated the perspectives of the host, ranging from a passive substrate to an integrated, coordinated organism. This range of perspectives can be valuable for characterizing the role of the host in the interplay between the components of the disease triangle. Using this approach, I characterized some complexities of specific plant-pest-environment interactions at a detailed level and captured mechanisms that cannot be detected at the broader scales.

I synthesized several of my findings into a disease trajectory that represents an individual plant-pathogen interaction. The trajectory incorporates results from the dissertation chapters and outlines the potential factors involved in the development of Cytospora canker disease (Fig. 5.1). There are two sides to the trajectory. The upper side represents factors that externally influence the interaction, and these external factors are both biotic (herbivory) and abiotic (temperature, water stress). The lower side of the trajectory represents internal factors, or host physiological mechanisms, that influence the plant-pathogen interaction.

The trajectory is initiated once the pathogen has infected the host, and the trajectory is then driven by paired internal and external factors. For example, following host infection by the pathogen, the host might experience water stress (an external factor) (Fig. 5.1). Water stress can increase disease susceptibility (Ch. 2, 3). However, the host

can actively respond to water stress and pathogen damage by increasing stomatal regulation of water loss and increasing water use efficiency (internal factors) (Ch. 3). This occurred despite disease damage to the sapwood, decreased water transport, and down-regulated photosynthetic parameters (reduced light saturated photosynthesis and light saturation point).

Another external factor which may affect the disease trajectory is foliar insect herbivory. If the host is colonized by the canker pathogen and then also experiences partial defoliation, then the host might compensate for the loss in photosynthetic leaf area by increasing leaf-area based water loss, and thereby increasing the potential for higher carbon uptake and assimilation (Fig. 5. 1). The higher potential for carbon gain in the host would likely be coordinated with increased hydraulic conductance and the maintenance of leaf water potential (Ch.4).

In summary, my study indicates that the right set of conditions (external factors) can increase susceptibility of *A. fruticosa* to the Cytospora canker disease. In addition to drought stress, there are several external factors (abiotic and biotic) that have the potential to affect Cytospora disease development on *A. fruticosa* in Alaska, including low temperature injury of buds (Helton 1961), frost injury (Reich and van der Kamp 1993), branch breakage (Stanosz *pers. comm.*), hare browsing (*pers. obs.*), and foliar herbivory (USDA 2009). However, my studies also indicate that *A. fruticosa* has physiological mechanisms in place to maintain homeostasis following biotic damage to the sapwood or leaf area. These mechanisms could ameliorate the short-term

physiological consequences of biotic pest damage. The physiological responses outlined in the disease trajectory are not widely recognized in plant disease literature and are not included as part of the classical disease triangle. Yet, these host responses could change the trajectory of disease development and affect disease outcome.

The disease trajectory characterizes some details of specific plant-pest-environment interactions. However, the trajectory that I present is not isolated from larger-scale processes. The dynamics of individual plant-pest interactions are nested within and influenced by processes at multiple levels: global, regional, and population (Fig. 5.2). Global climate change is the backdrop against which all levels of plant-pest dynamics can be evaluated. Global change directly drives changes to the regional climate, which in turn drives changes in the populations of hosts and pests. For example, my study takes in the boreal forest, which has been warming and drying for the last several decades (Barber et al. 2000, Oechel et al. 2000), and where both host and pest populations appear to be responding to regional climate change (Juday et al. 2005, Jepsen et al. 2008, Netherer and Schopf 2010). Nested within population-level responses to warming are individual interactions between plants and their pests. The challenge in studying plant-pest dynamics is in evaluating how these interactions are affected at these multiple levels, and then incorporating these scales into broader predictions for a specific plant-pest system under the warming climate.

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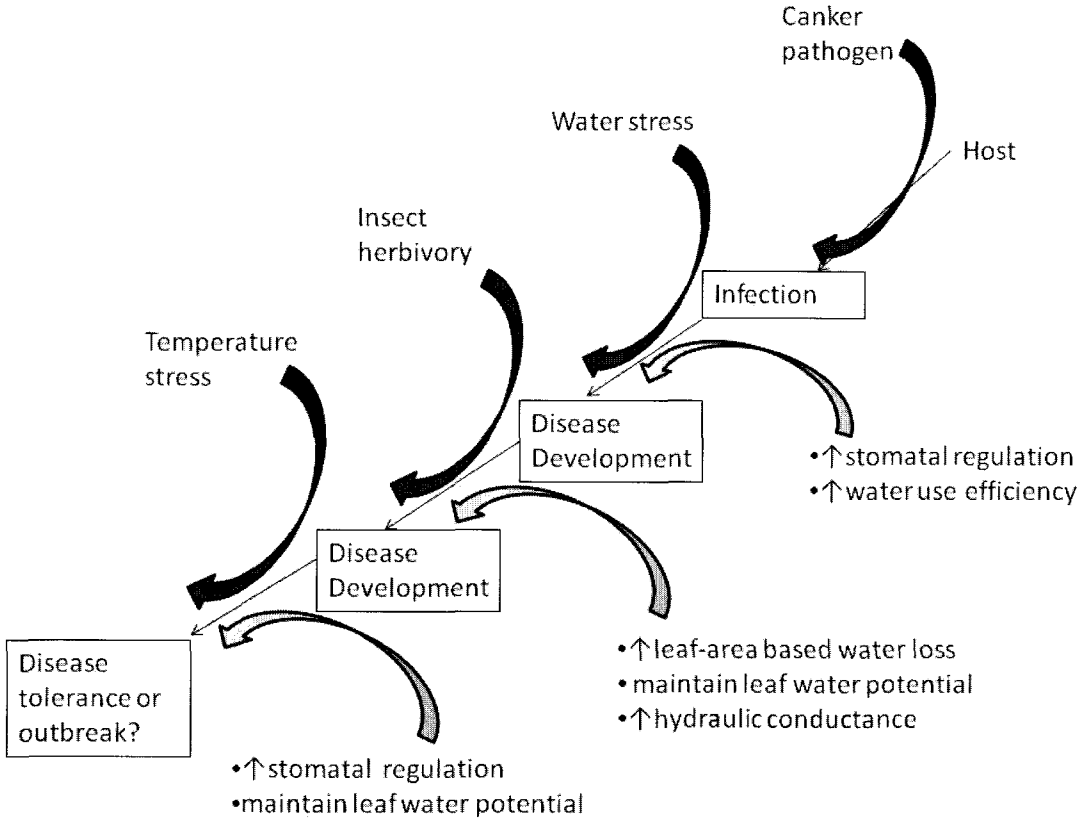

Figure 5.1 The disease trajectory. The upper part of the trajectory represents external factors and the lower part represents internal factors. All host responses outlined in this disease trajectory are physiological responses that were measured to the specific external stressors shown. gs: stomatal regulation; WUE: water use efficiency; PS: maximum photosynthetic rate; ψL: midday leaf water potential.

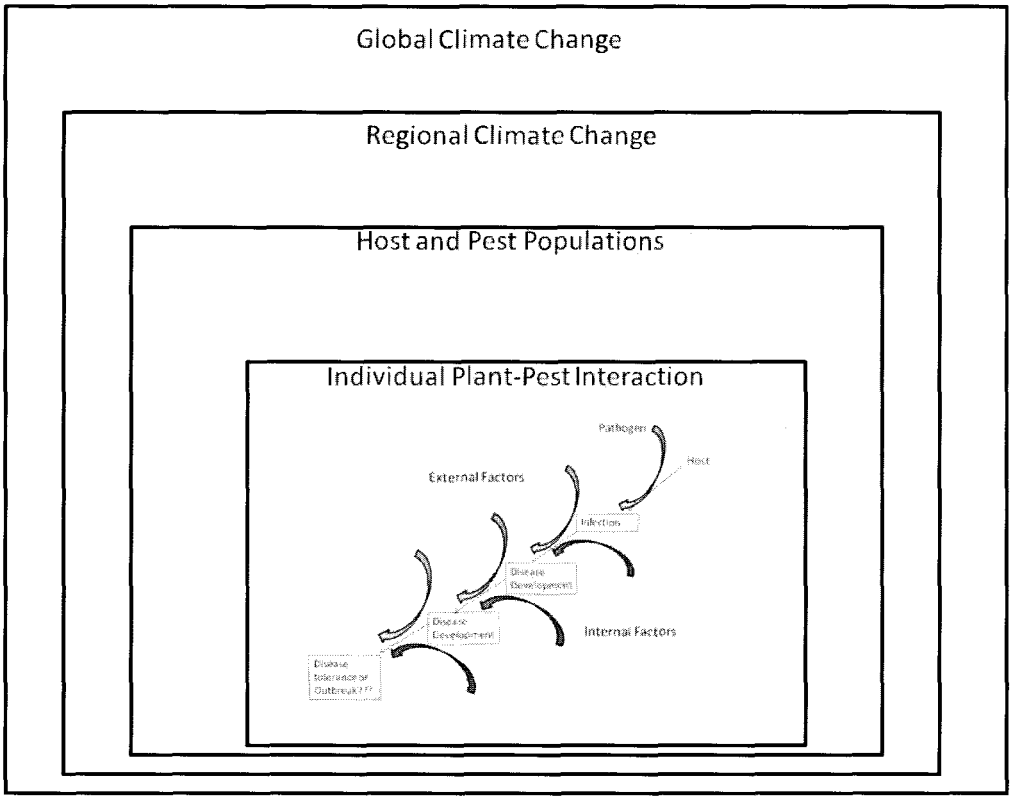

Figure 5.2 The disease trajectory nested within multiple scales. The dynamics of individual plant-pest interactions are nested within and influenced by processes at multiple levels: global, regional, and population.